Aspects of Vitamin D

Prevalence of deficiency and impact on musculoskeletal parameters

ANNE BJÖRK
Vitamin D is central in calcium turnover, and adequate levels are important for skeletal health. It is not clear how large contributions from food and sunlight are in Swedish primary care patients, considering the low radiation of UVB in Sweden and fortification of some foods, and whether differences exist between patients of immigrant and Swedish origin. Increasing incidence of osteoporosis-related fractures is a major global health problem. Genetic variations in metabolising enzymes and in the Vitamin D receptor \((VDR)\) have also been shown to be of importance to the overall effect of vitamin D. Polymorphic variation in the gene \(CYP2R1\) encoding the 25-hydroxylation has previously been reported to correlate with circulating levels of 25(OH)D3. Results of association studies between genetic variants of the \(VDR\) and muscle strength, as well as falls have been contradictory.

The purposes of this thesis were to examine possible differences in plasma-25(OH)D3 levels and intake of vitamin D between Swedish and immigrant female primary care patients, to estimate what foods contribute the most, and to identify contributors to vitamin D status (Paper I-II). Furthermore, the relationship between polymorphisms in the \(CYP2R1\) gene and levels of 25(OH)D3 as well as other biochemical parameters (parathyroid hormone, calcium, phosphate and fibroblast growth factor 23) of skeletal homeostasis, bone mineral density and incidence of fractures was investigated (Paper III). Also, the association between genetic variations in the gene for the vitamin D receptor and measures of muscle strength, physical performance and falls (Paper IV), was investigated by using data from a Swedish multicenter study of elderly men (MrOS).

Most important results: Vitamin D deficiency was common, with significant difference between Swedish born and immigrant patients (Paper I). Food intake of vitamin D is associated with circulating vitamin D, but the factors most strongly affecting vitamin D levels were reported sun holiday and origin (Paper II). \(CYP2R1\) polymorphisms are associated with circulating levels of 25(OH)D3 and bone mineral density (Paper III). \(VDR\) genetic variants do not appear to have a direct effect on muscle strength or physical performance and incidence of falls in elderly Swedish men (Paper IV).

**Keywords:** Vitamin D, \(CYP2R1\), Vitamin D receptor Gene, Polymorphisms, Muscle Strength

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To my sons
List of papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals:


III Björk, A., Mellström D., Ohlsson C., Karlsson M., Mallmin H., Johansson G., Ljunggren Ö., Kindmark A. Polymorphisms in the *CYP2RI* gene are associated with 25(OH)D3 and bone mineral density, but not with calcium and phosphate concentrations (MrOS Sweden). *Submitted.*

IV Björk A., Ribom E., Johansson G., Scragg R., Mellström D., Grundberg E., Ohlsson C., Ljunggren Ö., Kindmark A. Genetic variations in the vitamin D receptor gene are not associated with measures of muscle strength, physical performance and falls in elderly men. Data from Mr OS Sweden. *Manuscript.*

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<table>
<thead>
<tr>
<th>Abbreviation</th>
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<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>BMD</td>
<td>Bone Mineral Density</td>
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<td>BMI</td>
<td>Body Mass Index</td>
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<td>CV</td>
<td>Coefficient of Variation</td>
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<td>DNA</td>
<td>Deoxy ribonucleic acid</td>
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<td>DXA</td>
<td>Dual energy X Ray Absorptiometry</td>
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<td>FGF 23</td>
<td>Fibroblast Growth Factor 23</td>
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<td>FFQ</td>
<td>Food Frequency Questionnaire</td>
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<td>GLM</td>
<td>General Linear Model</td>
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<td>GP</td>
<td>General Practitioner</td>
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<td>LD</td>
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<td>P</td>
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<td>QCT</td>
<td>Quantitative Computed Tomography</td>
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<td>RIA</td>
<td>Radioimmunoassay</td>
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<td>SNP</td>
<td>Single Nucleotide Peptide</td>
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<td>UVB</td>
<td>Ultra Violet Radiation Type B</td>
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<td>UTR</td>
<td>Untranslated regions</td>
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<td>VDBP</td>
<td>Vitamin D Binding Protein</td>
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<td>VDR</td>
<td>Vitamin D Receptor</td>
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<td>WHO</td>
<td>World Health Organization</td>
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<tr>
<td>1,25(OH)2D3</td>
<td>1,25-dihydroxyvitamin D</td>
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<td>25(OH)D3</td>
<td>25-OH Vitamin D</td>
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“Why should you be interested in vitamin D?”
This is the question that my future supervisor, Gunnar Johansson, asked me when I told him I wanted to start a research project on vitamin D. Indeed, the knowledge about vitamin D deficiency as the cause of rickets and osteomalacia is almost 100 years old. Since the 1950s, Swedish children have been receiving vitamin D drops through national health care in order to prevent rickets because of low vitamin D production in the wintertime. So, the problem seemed to be solved, and rickets was almost eradicated in Sweden.

But I had read an article entitled “Vitamin-D deficiency maybe more common than we thought” in the Swedish medical journal (Läkartidningen), and became interested.

From the beginning, my interest focused on the possible importance of deficiency of vitamin D as a cause of depression which is common during the long winters in Sweden. But later on, my perspectives widened as I realized that immigrants in Sweden seemed to have very low levels of vitamin D in the blood. I became curious as to whether this would be an issue for general practitioners like myself when meeting patients in a Swedish health care centre. As a primary health care doctor, it is very common to meet patients with several diffuse symptoms. Often, we do not find any clear cause even though we examine the patients carefully.

As my work continued, interest in vitamin D increased enormously all over the world, thousands of articles were published, and research has now become more and more focused on mechanisms at the genetic level. I could never have expected that my project idea would take me to so many places, and all the interesting and wonderful people I would get to know. Starting off at Gottsunda health care centre in Uppsala, the national research schools in Family medicine and Osteoporosis took me further away to Umeå, Gothenburg and Linköping. My supervisors helped me attend a course in Bergen, Norway, and to take part in the vitamin D workshop in Delft, Belgium. Thanks to grants from the Swedish Medical Association and the Swedish Society of Medicine I was also able to attend conferences in Houston and London and finally, the national research schools in family medicine and osteoporosis enabled me to spend a 3 months’ pre-doc stay at the University of Auckland in New Zealand.

I have learnt so much and I am still interested in vitamin D. Hopefully; this thesis may lead to further knowledge.
Introduction

Vitamin D is essential to the human body in several ways. It is necessary for the maintenance of bone health (in the bones and the teeth), and is also essential to the regulation of calcium levels in the blood. Apart from these functions, knowledge has emerged in recent years about other possible ways in which vitamin D may be of importance. Research in recent decades has shown a possible correlation between vitamin D deficiency and a range of diseases such as diabetes, depression, coronary heart disease, cancer, psoriasis and multiple sclerosis.

History

Early life forms (phytoplankton and zooplankton), which have existed in the oceans for more than 500 million years, produced vitamin D when exposed to sunlight, and early cave paintings have indicated the appreciation of warmth and life-giving properties of the sun. Later on, during the industrial revolution in northern Europe, people moved to the cities and started living in houses that were built close to each other. In addition, the atmosphere was polluted by the burning of coal and wood. This resulted in children living with little direct exposure to sunlight, and several physicians during this time (Glissen, De Boot and Whistler) recognized that children living in industrialized cities demonstrated growth retardation and skeletal deformities, such as bony projections along the rib cage, (rachitic rosary), and either bowed legs or knocked knees.

Some years later, the cause of rickets was found to be lack of sunshine. But it was not until 1919 that vitamin D was discovered and its role in preventing rickets.

In Sweden, rickets, or engelska sjukan (“the English disease” as it was called in Sweden), was known in the middle of the 18th century. In a Swedish medical textbook from the mid-20th century, the cause of the disease was given as a »lack of fresh air, humid and dark housing and artificial feeding».

From the 1920s, cod liver oil was used to treat rickets. Since the 1950s, Swedish babies have been treated with drops containing vitamin D. Margarines, non-fatty milk and milk products have been fortified with vitamin D. After this, vitamin D deficiency became rare, and only occasional cases were seen. In the 1990s only a few cases of rickets were reported, in a few children.
raised on a vegan or nutritionally insufficient vegetarian diet without vitamin D drops and in three children from the Balkans, probably also because of lack of preventive treatment \(^5\).

**Vitamin D**

Vitamin D is not a single substance. Variants of vitamin D belong to a group of prohormones, which are necessary for the uptake of calcium in the intestine \(^1,6-8\). The most important forms are vitamin D\(_3\) (also known as cholecalciferol) and vitamin D\(_2\) (ergocalciferol).

Vitamin D\(_3\) and D\(_2\) can be ingested from the diet and from supplements. Vitamin D\(_3\) can also be produced via ultraviolet (UV) radiation of the skin. Since very few foods contain vitamin D, synthesis of vitamin D (specifically cholecalciferol) in the skin is the major natural source of the vitamin globally. But since production of vitamin D\(_3\) in the skin is dependent on UV radiation, intake through food and supplements is of greater importance in countries like Sweden, Norway, Finland and Canada, with low levels of UV radiation of the necessary wavelengths during part of the year.

**Production of vitamin D through ultraviolet radiation**

Vitamin D is obtained from the skin upon exposure to UV radiation. The length of effective UV exposure depends on the angle of the sun and atmospheric pollution, and the sun needs to be above 35° to be effective. Exposure of the skin to sunlight (the UVB band with wavelengths of 290 nm–315 nm) is needed for the photo-conversion of 7-hydroxy-cholesterol to pre-vitamin D\(_3\), which is then converted to vitamin D\(_3\). The amount of vitamin D\(_3\) produced depends on several factors such as exposed skin surface, season, latitude, skin pigmentation, and age \(^9\). Because of seasonal variation in UVB radiation, cutaneous vitamin D production is absent during part of the winter in Scandinavian countries, and the length of the ‘vitamin D winter’ increases with latitude. The skin synthesizing process is also dependent on use of sun screen and air pollution \(^10\). At the northern latitude of 60°, which is where Uppsala is situated, no vitamin D is produced in the skin between October and March.
Intake through food, supplementation and fortification

In countries where levels of UVB is low, the population is likely to be dependent on dietary sources of vitamin D to meet their biological needs during part of the year. Therefore, the authorities of some countries (Sweden, Finland, Canada) at high latitudes require fortification of certain food products. In Sweden, low-fat milk products and margarine are fortified. Fortification of food varies between the Scandinavian countries 11.

The two forms of vitamin D in food are found in fatty fish, egg yolks, meat products (vitamin D3), wild mushrooms (Vitamin D2) as well as in fortified products, such as milk and margarine 12.

When intake through food and production in the skin is not sufficient, vitamin D can be supplemented by ingestion orally (tablets and mixtures) or by injection.
Metabolism

Vitamin D status is usually defined by the concentration of calcidiol (25-hydroxy vitamin D (25(OH)D3) because this form reflects total body storage and is the precursor to the activated metabolite, 1,25-dihydroxyvitamin D (1,25(OH)2D3) 13.

Vitamin D3 is biologically inert and must undergo two hydroxylations in the body for activation. The first hydroxylation step occurs in the liver, where cholecalciferol produced in the skin or ingested via the diet (food and/or supplements), is hydroxylated in the liver to form 25(OH)D3. This reaction is catalysed by the microsomal enzyme vitamin D 25-hydroxylase (cytochrome P450 2R1, also called CYP2R1). 25(OH)D3 is then released into the blood. The circulating form (25(OH)D3), may then in a second step, be converted into calcitriol (1,25(OH)2D3) in the kidneys, released into the bloodstream and transported to various target organs. Vitamin D3 can also be metabolized by the cholesterol side chain cleavage enzyme, CYP11A1 to produce 20-hydroxyvitamin D and several other compounds. The significance of these compounds to the function of vitamin D is as yet unclear 14.

A variety of factors, including serum phosphorus and PTH, regulate the renal production of 1,25(OH)2D3. 1,25(OH)2D3 regulates calcium metabolism through interactions with its major target tissues, i.e. kidney, bone and intestine. 1,25(OH)2D3 also decreases further formation of vitamin D by enhancing the expression of 25(OH)D3 24-hydroxylase (24-OHase). It is believed that 25(OH)D3 is also metabolized in other tissues for regulation of cellular growth 15. (Fig.2)

CYP2R1

CYP2R1 or vitamin D 25-hydroxylase is a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases
which catalyse many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. Found in the liver, this enzyme converts vitamin D into 25-hydroxyvitamin D (calcidiol, 25(OH)D3), (which is the major circulatory form of the vitamin). Recent data suggest that CYP2R1 is the major but not exclusive 25-hydroxylase and that other enzymes might also be involved in this step of vitamin D activation\textsuperscript{16}. Polymorphisms of the gene for CYP2R1 have previously been shown to influence the levels of 25-OH vitamin D (25(OH)D3), and genome-wide significance for associations has been shown for the rs10741657 single nucleotide polymorphism\textsuperscript{17}.

\textit{Figure 3}. Schematic diagram of cutaneous production of vitamin D and its metabolism and regulation for calcium homeostasis and cellular growth. Figure from Holick 2004: Sunlight and vitamin D for bone health and prevention of autoimmune diseases, cancers, and cardiovascular disease. Reproduced with the permission of The American Society for Nutrition.
The Vitamin D receptor

The discovery of the vitamin D receptor (VDR) in different tissues has led to an increased interest in this vitamin, since this implies the possible influence on body tissues other than bone. The VDR is known to be involved in the effect of vitamin D on cell proliferation and differentiation. This receptor is principally located in the nuclei of target cells. Binding of calcitriol to the VDR allows the VDR to act as a transcription factor that modulates the gene expression of transport proteins which are involved in calcium absorption in the intestine. VDRs are expressed by cells in most organs, including the brain, heart, skin, gonads, prostate, and breast. The activation in the intestine, bone, kidney, and parathyroid gland cells leads to the maintenance of calcium and phosphorus levels in the blood, with the assistance of parathyroid hormone and calcitonin, and to the maintenance of bone content.

Vitamin D deficiency and insufficiency

Bone health

Vitamin D deficiency is currently defined as serum level 25(OH)D3 less than 25 nmol/L (10 ng/mL). The reason for this limit is that below this concentration, osteomalacia and rickets are commonly observed. There is also a general consensus that in adults with osteoporosis or osteopenia that 25(OH)D3 levels should be maintained at concentrations above 75 nmol/L.

Rickets

Rickets was defined as a specific medical condition in the 17th century and the first clear descriptions of rickets were published between 1645 and 1668, successively by Whistler, Boot, Glisson and Mayow. Whistler described rickets by reporting the musculoskeletal function as a combination of “flexible, waxy bones and flabby, toneless muscles” in young children.

Osteomalacia

Adults with vitamin D deficiency display defects in bone and muscle, characterized by osteomalacia (i.e. reduced bone mineral) and type 2 muscle fibre atrophy. Osteomalacia is caused by defective bone mineralization secondary to inadequate amounts of available phosphorus and calcium, or because of overactive resorption of calcium from the bone as a result of hyperparathyroidism. Muscle weakness and achy bone pain are the major sign and symptoms. Pseudo fractures seen on X-ray images are pathognomonic for osteomalacia. In uncertain cases, the most reliable way to establish the diagnosis is with a bone biopsy.
Vitamin D and non-skeletal diseases

Controversy exists on the definition of vitamin D insufficiency for the general population, about which target levels that should be achieved. The Institute of Medicine (IOM) in the United States has set the cut-off at 50 nmol/L. The IOM argues that higher levels have not been shown consistently to confer greater benefits, challenging the concept that “more is better”, that the prevalence of vitamin D inadequacy in the North American population has been overestimated, and that emerging evidence identifies risk for some outcomes at serum 25(OH)D3 levels above 125 nmol/L (50 ng/mL) 9.

Others consider blood levels below 75 nmol/L to be insufficient 25. The primary argument for the latter definition is based on the finding that serum PTH, which is inversely related to serum 25(OH)D3, decreases as serum 25(OH)D3 increases and reaches a plateau at a serum 25(OH)D3 of approximately 75 nmol/L (30 ng/ml). However, this has been regarded by some as controversial, since different studies have shown a large variation in the plateau level of PTH ranging from a serum 25(OH)D3 level of 45 to 75 nmol/L (18 to 30 ng/ml) 26, 27.

The reason why the definition of vitamin D insufficiency is of major importance is because it subsequently affects the recommended dose of vitamin D intake, assuming that all intake would be only through food and supplements. In that case, to increase the level in the general population people would be required to take a dose of approximately 800 IU daily, whereas a minimum level of 75 nmol/L would require a daily dose of approximately 4000 IU/day.

Measurement of vitamin D status

The problems with measurement of 25(OH)D3 in the blood are attributable to the molecule itself, since this compound is very hydrophobic and also exists in two forms; 25(OH)D2 and 25(OH)D3. The lipophilic nature of 25(OH)D3 makes it especially vulnerable to matrix effects in all protein binding assays (PBA) 28.

The first methods developed for measurement of 25(OH)D3 were relatively cumbersome 29, and the knowledge related to vitamin D metabolism has increased enormously since newer methods have been introduced. HPLC (High Pressure Liquid Chromatography) is considered to be the golden standard. Other methods are now available. A new generation of immunoassays have proven useful for routine diagnostic purposes in clinical laboratories 30. In order to ensure the quality of vitamin D measurements, laboratories can take part in DEQAS, Vitamin D External Quality Assessment Scheme 31.
Immigration and food habits

At the beginning of 2016, approximately 17% of the inhabitants in Sweden were born in another country 32. The proportion originating from countries at lower latitudes (the Middle East and Africa) has increased. Another 4% have an immigrant background, meaning that they were born in Sweden but both their parents were born in another country.

Food habits vary between different population groups. Dietary habits in migrant populations are likely to become less healthy because of an increased exposure to Western-style fast foods. Immigrants from the third world are more prone to acquire nutritional deficiency diseases, such as rickets, osteomalacia and iron deficiency anaemia than the rest of the population in the recipient countries 33.

Studies on immigrants from Pakistan, Turkey, Somalia, Sri Lanka, Iran and Vietnam in Denmark, Norway and Sweden have demonstrated a high prevalence of vitamin D deficiency, with the lowest levels in women 34-36, 37.

Osteoporosis

Osteoporosis is a skeletal disease characterized by low bone mass and micro-architectural deterioration of bone tissue with a consequent increase in bone fragility and susceptibility to fracture 38. The bone mineral density (BMD) is reduced, bone microarchitecture deteriorates, and the amount and variety of proteins in bone are altered. This leads to degeneration of bone tissue, a reduced bone mass, consequently an increase in bone fragility and risk of fracture.

The World Health Organization (WHO) previously defined osteoporosis based on BMD. A standardized score, called T-score, comparing BMD to average values for young healthy women was used to define the disease 39. However, a revised description of osteopenia and assessment of osteoporosis was released in 2008, using BMD together with selected risk factors for fracture, height and weight 40.

The incidence of osteoporosis-related fractures with increasing age is a major health problem, leading to economic problems for both the individual and society, as well as suffering and increased mortality.

Aging is associated with large changes in bone mass and architecture, in men as well as in women 41. The exponential increase in fracture incidence as men age is as dramatic as the similar increase that occurs in women, but it begins 5-10 years later in life 42.
Bone mineral density measurements (bone densitometry)

Measurements of BMD are used to determine bone strength and loss of bone mass in order to determine the presence of osteoporosis or not, and to assess the risk of fracture. The basic principle of dual energy X-ray absorptiometry (DXA), which is the most common method used, is a painless, non-invasive technique with low radiation exposure. The DXA technique gives measurements of areal density, as opposed to quantitative computed tomography (QCT) which measures volumetric density 43, 44.

Genetics

DNA

DNA is composed of building blocks made up of the nitrogenous bases Adenine, Cytosine, Guanine and Thymine, commonly abbreviated A, C, G and T. DNA is in turn composed of combined stretches of these four bases attached to a backbone of sugars and phosphate groups. Two DNA strands together form anti-parallel double helices, making up the chromosomes. Genomes are composed of several chromosomes that reside within the nucleus of almost every cell type in the body. There are two copies of each chromosome, one derived from each parent. Numerous interindividual variations (polymorphisms) which are present in our genomes, separate each of us genetically from our fellow humans.

Polymorphisms

Polymorphisms are one type of DNA sequence variation in the human genome. A polymorphism is a type of variation which is common in the population and usually does not cause a disease on its own, but may be involved in influencing characteristics such as height and hair colour. However, these common sequences can also predispose, together with other gene variants, to common diseases and influence the health. The most common type of polymorphisms is the single nucleotide polymorphism (SNP).

An SNP is a substitution at a specific genomic position, where more than one nucleotide is represented in the population. In order to be defined as a SNP, the least abundant single nucleotide variant has to occur in the population at a frequency higher than 1%. If the frequency is lower, the allele is regarded as a mutation. When two human chromosomes are compared, they differ due to a SNP on average once every 1000-2000 nucleotides. The human genome contains roughly 10 million SNPs 45.

Most SNPs are found in the non-coding regions, such as within introns or in intergenic regions, and do not directly affect the function of the specific
protein. So called functional SNPs may be coding, or may affect transcription levels or mRNA stability.

SNPs lying physically close on a chromosome are often associated with each other, a phenomenon known as linkage disequilibrium (LD). The combination of variants along a chromosome is known as a haplotype, where adjacent SNPs can be inherited together and constitute haplotypes. The human genome can be grouped into haplotype blocks which are highly correlated across populations. A haplotype map of the human genome has been constructed by the Hap Map project, from populations with ancestry from parts of Africa, Europe and Asia. After the initial efforts in the HapMap project ended, the 1000 genomes project has become the reference dataset for global haplotype mapping, with a goal to find most genetic variants with frequencies of at least 1% in the populations studied.

Genotyping and haplotypes
Genotyping is the process used to determine which genetic variant is present at a certain position in the genome. DNA sequencing can be used for this purpose, as can techniques only giving information about variable positions of the genome. It has been observed that certain variable positions that are physically close on a chromosome have a tendency to occur together. Adjacent SNPs can be inherited together and these co-occurrences, consisting of combinations of variants along a chromosome, are known as haplotypes. The haplotypes are useful because the genotyping of a limited number of polymorphisms can be sufficient to know the full repertoire of genetic variation in a genomic region. Haploblocks are highly correlated across populations, and a haplotype map of the human genome has been constructed by the Hap Map project, from populations with ancestry from parts of Africa, Europe and Asia.

Effects of VDR polymorphisms
Polymorphisms of VDR have been shown to be associated with bone mineral density and fracture risk. The VDR gene is also regarded as an important candidate gene in muscle function for several musculoskeletal phenotypes. However, results of these investigations are considerably inconsistent and hardly comparable because of the multiple types of muscle strength measurements and the stratified study populations. Several association studies on the VDR gene and muscle strength have been done. VDR genotypes have in some cases been shown to be associated with differences in muscle strength, whereas in other studies no proof of association could be found.

Controversies also exist in the literature as to whether vitamin D’s effects in the musculoskeletal system are direct via local VDR signals or indirect via its systemic effects in calcium and phosphate homeostasis. Observational
studies, mainly in older populations, have indicated that vitamin D status is positively associated with muscle strength and physical performance, and inversely associated with the risk of falling. Also, clinical trials of vitamin D supplementation in older adults with low vitamin D status have reported improvements in muscle performance and reductions in falls 54.

The underlying mechanisms have been assumed to be partly through direct activation of the VDR by 1,25(OH)2D3, and partly indirectly through calcium and phosphate 55.
Aims

General aims
The overall aim of the studies included in this thesis is to determine prevalence and underlying causes of vitamin D deficiency in primary care patients, to identify contributions to vitamin D concentrations in the blood, and to investigate the impact of polymorphic variations in genes related to vitamin D metabolism on musculoskeletal parameters.

Specific aims

I  To investigate possible differences in plasma-25(OH)D3 levels and intake of vitamin D between Swedish and immigrant women in the same primary health care centre.

II To measure plasma concentrations of 25(OH)D3 and to identify contributors to vitamin D status in Swedish women attending a primary health care centre at latitude 60°N in Sweden.

III To study differences in vitamin D status, biochemical markers, BMD and fracture incidence in individuals with different genetic variations of the enzyme CYP2R1 in a cohort of 3014 elderly Swedish men (MrOS Sweden).

IV To investigate possible differences in vitamin D status and muscle strength in subjects with different variants of VDR, using data from MrOS Sweden.
Materials and methods

This thesis is based on four papers. The first two papers are from a primary health care study of women aged 18 to 75 years. The third and fourth paper are from a large osteoporosis study of elderly men. An overview is presented in Table 1.

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<th>Data collection method</th>
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<td>Study I Cross-sectional, observational study.</td>
<td>Analysis of blood samples, Individual questionnaires.</td>
<td>61 female patients 18-75 years at a primary health care centre.</td>
<td>Descriptive and comparative statistical Analyses.</td>
</tr>
<tr>
<td>Study II Cross-sectional, observational study.</td>
<td>Analysis of blood samples, Individual questionnaires.</td>
<td>61 female patients 18-75 years at a primary health care centre.</td>
<td>Descriptive and comparative statistical analyses. Regression analysis</td>
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Study populations and Samples

Study I and II

**Primary health care patients**

This study was conducted in Uppsala, a medium-sized city in Sweden, and performed between January and March 2009. 31 immigrant and 30 Swedish-born female patients attending the primary health care centre (irrespective of the reason for the appointment) were recruited. Four of the patients who were invited to participate chose not to participate, owing to lack of time and interest. The primary health care centre is located in a district of the city, where half of the inhabitants have an immigrant background, with two thirds of them originating from the Middle East.

The study was carried out in co-operation between a dietician and a general practitioner. Inclusion criteria were female patients born in the Middle East or Africa aged 18-75. Immigrant women attending the primary health care centre were consecutively, irrespective of reason for the appointment, were consecutively asked if they would be willing to participate in a study concerning vitamin D. Those who were interested in participating received both oral and written information and signed a written consent form.

Exclusion criteria were current pregnancy, current breastfeeding or current treatment with vitamin D.

Study III and IV

**The Swedish MrOS Cohort**

The MrOS (The Osteoporotic Fractures in Men) study is a multicentre prospective fracture epidemiology investigation involving elderly men from different sites around the world, including Hong Kong, Sweden and the US. The Swedish part consists of 3014 men aged 70 years or older. Participants were recruited at each of three academic medical centres: Sahlgrenska Academy in Gothenburg, Malmö General Hospital and Uppsala University Hospital.

The study participants were randomly selected from the national population register and invited by mail. 45% of those contacted participated (n=3014), constituting three sub cohorts in three cities: Malmö (n=1005), Göteborg (n=1010), and Uppsala (n=999).

Eligibility for study participation required being between 70 to 80 years of age and the ability to walk unassisted by another person, to provide self-reported data, and to understand and sign an informed consent, and not to have bilateral hip prostheses. Informed consent was obtained for all subjects.
Methods

Common for study I and II:

Subjects were interviewed by a dietician and a general practitioner (GP) concerning their general health, current smoking habits, education, eating habits, holidays in the sun, use of sunscreen and skin reactivity. Data were obtained by structured written questionnaires, oral interviews and observation. Height and weight were measured and Body Mass Index (BMI, kg/m²) was calculated. Blood samples were drawn and sent for analysis to the laboratory. In six cases a professional interpreter was used and in one case, the husband was present and helped to translate. A questionnaire previously used by Burgaz\textsuperscript{57}, which included aspects of UVB exposure, such as duration of sunlight exposure, tanning-bed use, frequency of sun vacations during winter (i.e. travel to a latitude with high UVB radiation during the wintertime with the aim of sunbathing), and also included estimation of photosensitivity according to Fitzpatrick sun-reactive skin-type classification which includes six different skin types, was used\textsuperscript{58}. Skin types I to III include light skin, while skin types IV to VI include darker skin. Intake of vitamin D from food and supplements was estimated using a new semi-quantitative food frequency questionnaire (FFQ), designed for this study, consisting of 15 foods and 8 frequencies and aimed to assess the intake during the previous two to three months. The FFQ included foods containing vitamin D naturally, and foods fortified with vitamin D (low fat milk, milk products and margarine). The feasibility of the FFQ was checked with the patients and all items in the FFQ were discussed together with each participant during the appointment.

Plasma-25(OH)D\textsubscript{3} was analysed using an automatized immunochemical method (LIAISON\textsuperscript{†} (Diasorin)). Plasma calcium was analysed at the clinical chemistry laboratory, University Hospital in Uppsala, using standard methods.

Common for study III and IV:

Baseline data regarding serum parameters and BMD, and data on osteoporotic fracture incidence from the 5-year follow-up were analysed.

At the clinic visits, participants completed questionnaires regarding medical history, current medication use, and lifestyle characteristics. All tests were performed and registered by research nurses or trained research staff according to standardised protocols. Blood (n=2961) and serum samples (n=2908) were collected and stored at -20 °C and -70 °C, respectively, until processing and analysis.
Anthropometry
Height and weight were measured using an electric scale or balance beam scale and a Harpender stadiometer. Body mass index (BMI) was calculated as weight (kg) divided by height (m) squared.

Serum measurements
25(OH)D3 concentrations in serum were measured by Nichols Advantage automated assay system (San Juan Capistrano, CA, USA). The inter-assay CV was 15–16% at all 25(OH)D3 levels.
Phosphate, calcium and albumin were analysed at the respective hospitals department for clinical chemistry using standard methods. Estimated glomerular filtration rate (eGFR) in ml/min/1.73 m² was calculated from serum cystatin C (Cystatin C Immunoparticles, Dako A/S, Glostrup, Denmark) according to the formula 79.901*(Cyst C [mg/L])^{-1.4389}. Intact PTH was measured by a second generation immunometric assay, Immulite 2000, (Los Angeles, USA). 25(OH)D3D levels were measured by Nichols Advantage automated assay system (San Juan Capistrano, CA, USA). Serum concentration of intact FGF23 was analysed in using a two-site monoclonal antibody based ELISA (Kainos Laboratories International; Tokyo, Japan).

Study III
BMD measurements
BMD of the lumbar spine, total hip and femoral neck was measured using DXA scanners: Lunar Prodigy DXA (GE Lunar Corp., Madison, WI, USA) in Malmö and Uppsala and Hologic QDR 4500/ A-Delphi (Hologic, Bedford, MA, USA) in Gothenburg. DXA measurements performed with equipment from different manufacturers were converted to a standardized BMD.

Genotyping of the CYP2R1 gene
DNA was isolated from whole blood extracted at baseline, and was available from 2868 participants. Using a saturation approach representing HapMap SNPs and HaploView scoring, a total of 11 SNPs covering 100 kb of the genetic region surrounding the CYP2R1 gene including the 3’ and 5’ untranslated regions (UTRs) were selected. Genotyping was performed using the Sequenom Mass ARRAY® iPLEX Gold technology (Sequenom Inc., Newton, MA) by single base primer extension and MALDI TOF Mass Spectrometry.
Successful genotyping was obtained from 8 SNPs with overall call rate of 97.8%, covering 100 kb of the genetic region surrounding the gene encoding for CYP2R1 in the 2868 men with available samples of DNA. Allele frequencies were calculated and found to be in Hardy-Weinberg (HW) equilibrium in the cohort for all SNPs.
Haploview software version 4.2 was used to calculate linkage disequilibrium (LD) values, generate haplotype blocks and diagrams, as well as suggesting tagging SNPs using the tagger algorithm.

Study IV

Muscle strength and functional test measurements

Hand grip strength was measured with a Jamar® hydraulic hand dynamometer (5030J1, Jackson, MI, USA). The participant was seated in a standard chair with the arm resting on a moveable table with the dynamometer in an upright position. Two measures of each hand were collected, with the stronger of the two results (in kilograms of force), used in analyses. (Fig.4)

In the 6-m walking test and the 20-cm narrow walking test, participants followed a walking course laid out on the floor. In the first test, the participants walked 6 m at their usual pace. The duration of the walk was measured as well as the number of steps. The step count included both right and left steps, the initial starting step and the step that first touched the floor across the finish line. In the 20-cm narrow walking test, the participants walked the 6-m course within a 20-cm narrow path. Two scored trials were performed and time was scored if there were no more than two deviations from the path. The shortest time of the two scored trials was used in data analyses.

The timed stands test was performed in a straight-backed chair without arms, at a seat height of 45 cm. The time to complete five chair stand(s) without using the arms was recorded in order to assess the muscle endurance of several large muscle groups.
Falls
Self-reported falls during the 12 months preceding the measurements were evaluated as a part of the medical history review. Participants were asked, “Did you fall at any time during the past 12 months?” If so, they were asked how many times, which was recorded as 1, 2–3, 4–5 or 6+. Level of physical activity was measured using the Physical Activity Scale for the Elderly questionnaire.

VDR Genotyping
DNA was isolated from whole blood extracted at baseline, and was available from 2924 participants. A total of 8 SNPs of the VDR gene covering the gene including 3UTR, and 5 UTR were selected for genotyping, including the two preselected SNPs rs9729 and rs731236 defining haplotype blocks for previously genotyped SNPs in the VDR gene (TaqI, ApaI, and BsmI).

Allele frequencies for these SNPs were calculated and found to be in Hardy-Weinberg (HW) equilibrium in the cohort for all SNP. Haplowiev 4.2 was used to generate haplotype blocks, linkage disequilibrium (LD) values and diagrams, as well as tagging SNPs using the tagger algorithm.
Ethical approval

Study I and II:
Informed consent was obtained for all subjects and the study was approved by the local ethics committee at Uppsala University (Local ID number 2008:359).

Study III and IV:
Informed consent was obtained for all subjects and the study was approved by the local ethics committees (Dnr LU 693-00, Gbg M 014-01, Ups 01-057).

All four studies were performed in accordance with the Declaration of Helsinki.
Statistics

Paper I

An estimation of power carried out before the study, assuming 60% of vitamin D deficiency in the immigrant group and 20% in the Swedish group, indicated that samples from 60 women would be sufficient to reveal a statistically significant difference with 90% power using a two-sided test of significance and p < 0.05. Data were analysed with the SPSS (version 20.0) statistical programme packages. Neither the original data nor the log-transformed were normally distributed. Summary statistics such as medians, percentiles, and proportions were therefore computed using standard non-parametric methods. Differences in proportions were calculated with the chi-square test. Linear regression was used to evaluate the relationship between quantitative parameters. A p-value of <0.05 was regarded as a statistically significant difference.

Paper II

The same estimation of power as in paper I was used. The statistical analysis was performed using the SAS program package version 9.2 (SAS Institute, Cary, NC, USA). Continuous variables were age (years), BMI (kg/m2), weight (kg), vitamin D intake (food and supplements) (μg/24 hours) and plasma calcium (mmol/L). The categorical variables included origin (immigrant/native), education (<12 years/>12 years), use of sunscreen (no/yes), wearing veil (not observed/observed), sun holiday past year (no/yes), skin type (I-VI) and current smoking (no/yes). Non-parametric tests were used since the data was not normally distributed. Correlation was analysed with Spearman’s correlation coefficient and differences between the groups in continuous variables with Wilcoxon’s test. Differences in proportions were calculated with the chi-square test. The SAS procedure General Linear Model was used for univariate and multivariate analyses. To avoid model overload stepwise regression as well as backward elimination of non-significant variables were used. A p-value of <0.05 was regarded as a statistically significant difference.

Paper III

Statistical analysis was performed using the IBM SPSS program version 22 and Statistica version 13. The preselected SNPs were analysed for associations between 25OHD3 values and other biochemical parameters, as well as BMD. Differences between characteristics for the different SNPs were computed by ANOVA and Tukey’s post hoc testing. A p-value of <0.05 was regarded as a statistically significant difference. Values are given as mean ± SD unless otherwise stated. Probability for deviation from Hardy-Weinberg equilibrium (HWE) and major and minor allele frequencies were calculated using chi-
squared test for HW equilibrium for biallelic markers. Differences in relative fracture risk between alleles of tagging SNPs were compared by using chi-squared method. The analyses were done for osteoporotic fractures in all participants with data on genotype and fracture.

Paper IV
IBM SPSS Statistics (Version 24) was used in the statistical analysis. Data are presented as means, standard deviation (SD) or proportions (%). General linear regression analysis (ANOVA) linear regression and chi-squared methods were used. The Bonferroni method was used in order to account for multiple comparisons. A p-value of <0.05 was regarded as a statistically significant difference. Deseasonalised values for 25(OH)D3 were calculated by assuming that 25(OH)D3 follows a sinusoidal pattern, using a cosinor model fit to baseline measurements, as published in previous reports 62, 63. For comparisons of muscle strength and physical performance tests, the measured (non-deseasonalised levels) of 25(OH)D3 were used.
Results and discussion

In Paper I, the major finding was that in these Swedish primary care patients, vitamin D deficiency (plasma-25(OH)D3 <25 nmol/L) was significantly more common among immigrant women, (61%) than among Swedish women (6.7%), p <0.001 with linear regression. Previous results from studies of immigrants in Denmark and Norway had shown low levels of 25(OH)D3 in immigrants, but since fortification of food with vitamin D varies between the Nordic countries and these studies were of general population, we wanted to find out if deficiency was common in Swedish primary care patients attending Gottsunda primary care health centre. The fact that we met all patients during a personal visit with a dietician and a GP, and in cases where needed, also with an interpreter present, increased the possibility of more complete data acquisition and the inclusion of illiterate subjects.

The median level of 25(OH)D3 for the immigrant women in our study was 22.2 nmol/L, which was higher than in the Danish study by Andersen (12.0 nmol/L) 34, but lower than those found in Norway by Holvik 35. Reasons for these differences could be lower intake of vitamin D through food in the Danish women, that our samples were taken earlier during the year (Jan to March, when the 25(OH)D3 levels are lowest during the year), that Norwegians use cod liver oil (which is not common in Sweden), and differences in food fortification.

Another important result of our study was that daily intake in general of vitamin D through food was low, with only 3 Swedish subjects reaching the recommended level. Only 11 out of the 61 women took any kind of vitamin D supplements.

We hypothesized that the differences in 25(OH)D3 levels between women of immigrant and Swedish origin were due to differences in food habits, and statistical analysis showed indeed a positive correlation (r=0.35, p <0.01) between the intake of vitamin D through food and plasma-25(OH)D3 levels between the two groups. An interesting result was that the immigrants and the Swedish women obtained their vitamin D from different sources, with a lower estimated intake from fortified milk and margarine on bread and in cooking in the immigrant group than in the Swedish group, presumably because these foods are not common to their traditional habits.

The results could possibly have been affected by the choice of method for measuring 25(OH)D3, and another method, for example HPLC, which is the standard reference method might have given different results, with some of
the women having slightly higher levels. However, even if that would be the case, most of the immigrant women would have 25(OH)D3 levels below 50 nmol/L and the difference in plasma 25(OH)D3 between the two groups would still remain. In retrospect, it would have been of interest to have measured other parameters of calcium and phosphate metabolism, but this was not possible due to lack of funding. Repeated measurements of bone mineral Density (BMD) and follow-up of fractures would also have been of interest.

The relatively weak correlation ($r=0.35$) between 25(OH)D3 levels and intake of vitamin D through food inspired us in Paper II to evaluate other contributors to vitamin D status, using data from the same study group as in paper I. This study showed that reported sun holiday and ethnic Swedish status were the factors that positively, significantly and independently affected 25(OH)D3 concentrations in plasma, with sun holiday ranking the highest. We unexpectedly discovered insignificant effects of vitamin D intake from food and supplement as well as wearing of a veil. The importance of a sun holiday found in the present study is in line with the study by Burgaz 57 on elderly Swedish women from the general population, but a difference was that our study included younger patients, and from a primary health care setting. The effect of immigrant origin on 25(OH)D3-levels is harder to interpret. Genetic differences could be a partial explanation, but confounding factors could also be socioeconomic factors such as differences in income and education. This has indeed been shown to be a possibility in a recent paper of Abboud et al, which concludes that variations in the 25(OH)D3 levels achieved with relatively constant vitamin D input appear to be due to small contributions by genetic polymorphisms in genes involved in vitamin D and metabolite synthesis, transport and degradation, as well as lifestyle and body composition factors such as calcium intake, exercise and lean and fat mass 14.

Only one immigrant woman and half of the Swedish women had 25(OH)D3 levels above 50 nmol/L, which is considered a minimum level for bone health 64. At a follow-up contact (telephone or personal visit), the subjects were informed about these results. Those who had 25(OH)D3 levels below 50 nmol/L received advice on food rich in vitamin D, and the subjects with vitamin D deficiency (<25 nmol/L) were also prescribed a combination drug containing calcium 500 mg and cholecalciferol 800 IE. At a follow-up visit one year later, 23 (13 of the immigrant and 10 of the Swedish) out of 50 women (24 immigrant and 26 Swedish) still had 25(OH)D3 levels below 50 nmol/L. This shows the need for more investigations, in Swedish women of both native and immigrant origin.

In Paper III, the major finding was that genetic variation in 8 different SNPs of the gene for CYP2R1 correlate with levels of circulating 25(OH)D3, but were not associated with measures of calcium and phosphate homeostasis (calcium, phosphate, PTH and FGF23). This lack of correlation between these SNPs and the other laboratory parameters was unexpected, since vitamin D deficiency often causes secondary hyperparathyroidism, and therefore it
would be assumed that polymorphisms associated with low 25(OH)D3 levels would be associated to high PTH and calcium levels. A possible explanation could be that vitamin D deficiency (25(OH)D3 <25 nmol/L) was rare in the Swedish MrOS cohort. These findings are in agreement with other studies on subjects of European ancestry 17, 65, 66.

Analysis of data from BMD measurements showed higher BMD values for the GA allele of rs11023374 (p=0.01) in the hip and a higher BMD of the femoral neck (p=0.05) for the CC allele of the rs10832313 SNP. Intriguingly, in both cases, the BMD was higher for the SNP variants associated with lower levels of 25(OH)D3. A similar, but not significant tendency was shown for all other SNPs.

We also found no association in fracture incidence 5 years after baseline with any of the 8 SNPs, in concordance with the findings in a cohort of 342 subjects from Austria 65.

In retrospect, it would have been interesting to also have had access to measurements of vitamin D binding protein in order to calculate the bioactive concentration of vitamin D. In a future study, it would also be interesting to study subjects with vitamin D deficiency, as well as subjects of other ethnic origin for association between CYP2R1 variants and bone and calcium homeostasis.

In paper IV, genetic association analysis was performed for 8 Single Nucleotide Polymorphisms (SNPs), covering the genetic region surrounding the VDR gene in the 2924 men with available samples of DNA. The main results in this study were that VDR genetic variants are not correlated to muscle strength nor physical performance (hand grip strength right and left, 6 meters walking test (easy and narrow) and timed stands test in elderly Swedish men. These results are important, since previous studies have analysed smaller sample sizes and have shown contradictory results.

One of the findings in our study is that one of the 8 VDR SNPs of the gene for the VDR receptor, rs7136534, was associated with incidence of falls. The underlying mechanism for the association to this SNP is not clear.

It would have been interesting to have had more subjects with low levels (<25 nmol/L) of 25(OH)D3, as these were few in the group studied. Also, all participants were able to walk without assistance and were generally in good health. Most were of Caucasian descent. Therefore, generalizability to those less-mobile, institutionalized, other ethnic origins and to women may be limited.
Summary

Paper I

Hypothesis: There are differences in plasma 25(OH)D3 levels and intake of vitamin D through food between Swedish and immigrant female patients in primary health care.

Method: We measured levels of 25(OH)D3 in blood samples from 61 female patients (30 of Swedish and 31 patients of immigrant origin), at a primary health care centre in Uppsala. A modified food frequency questionnaire (FFQ) was used for estimating intake of vitamin D.

Results and Conclusion: A significant difference in circulating vitamin D levels was seen between Swedish and immigrant women (p <0.01). A correlation between 25(OH)D3 levels and food was seen (r=0.35). Analysis of food intake showed that immigrant women consumed less fortified milk and margarine but more meat. These results could be due to difference in food culture, but other factors explaining the difference in 25(OH)D3 levels could also be important.

Paper II

Hypothesis: Factors other than food contribute to 25-OH-vitamin D (25(OH)D3) levels in plasma.

Method: 61 female primary care patients were randomly selected, irrespective of reason for visiting the health care centre. Subjects were examined with blood samples and interviewed by a dietician and a general practitioner (GP). Questionnaires for sun habits and food frequency (FFQ) were used. Regression analyses were used with univariate and multivariate methods.

Results and Conclusion: Vitamin D deficiency (25(OH)D3 <25 nmol/L) was common (34.4%). Multivariate analysis showed that reported sun holiday of one week during the last year, at latitude below 40°N for the purpose of sunbathing, and native origin were significantly, independently and positively associated with 25(OH)D3 concentrations in plasma.
Paper III

Hypothesis: Polymorphisms in the CYP2R1 gene encoding Vitamin D 25-hydroxylase influence levels of circulating 25(OH)D3, calcium, phosphate, parathyroid hormone (PTH) and fibroblast growth factor (FGF23), as well as bone mineral density (BMD) and incidence of fractures.

Methods: Baseline data from the prospective population based cohort study of elderly men (MrOS) were analysed. Genotyping was performed for 8 SNPs covering the genetic region surrounding the CYP2R1 gene. Fracture data regarding incidence during 5 years were used.

Results and conclusions: Six of the eight SNPs were significantly associated with circulating levels of 25(OH)D3, but no correlations were found with levels of calcium, phosphate, PTH or FGF23 for any of the SNPs. A slight increase in BMD for two SNPs variants associated with lower levels of 25(OH)D3 was seen. No differences in fracture incidence between the polymorphisms were found. These results could be due to some compensatory hormonal regulation, or possibly a mechanism unrelated to vitamin D metabolism.

Paper IV

Hypothesis: Genetic variation in the vitamin D receptor (VDR) gene is associated with 25(OH)D3 D levels, muscle strength, physical performance and falls.

Methods: Baseline data from a prospective population based cohort study of elderly men (MrOS) were analysed. Genotyping was performed for 8 SNPs covering the genetic region surrounding the VDR1 gene in 2924 men. Five different phenotypes of muscle strength were studied, and a questionnaire was used regarding the incidence of falls during the 12 months preceding the measurements.

Results and conclusions: There was no significant association between genetic variation in the VDR and differences in muscular strength or physical performance. These results which were analysed in a large, well characterised cohort are important, since previous studies have used smaller sample sizes and have shown contradictory results. A difference in incidence of falls was shown between alleles of the SNP rs7136534. The underlying cause for this association needs to be investigated further.
Conclusions and future perspectives

The results of this thesis show that very low concentrations (<25nmol/L) of 25(OH)D3 were common in female primary care patients, with a predominance of women of an immigrant origin. If this is actually the case, that a large proportion of immigrant women in Sweden have signs of severe deficiency, this should be investigated further in terms of possible consequences to future illness.

The importance of interpreting single measurements of vitamin D and the need to take consideration seasonal variations into account, needs to be investigated further.

The effect of treating individuals with very low levels should be studied further, as most treatment studies previously performed have not been focusing on these individuals.

The importance of differences in food habits should be taken into account, and the selection of which foods that should be fortified should be discussed.

Furthermore, the fact that an even larger proportion of patients have levels below 50 nmol/L should be evaluated. The question of D vitamin prophylaxis should be discussed, whether it is necessary or not, as to which dosage that should be appropriate, as well as how long it should be provided.

The consequences of genetic differences in particular with regards to vitamin D and calcium-phosphate metabolism, as well as impact on various phenotypes should be further elucidated.

Another group that has not yet been studied much with regards to vitamin D deficiency is elderly immigrant men. Differences in genetic variations between these men and Swedish men, and the consequences to vitamin D deficiency, osteoporosis, and increased fracture incidence should be of importance, and of value for the future health situation in Sweden.

Possible associations between genetic variation, vitamin D status, muscle strength and incidence of falls need to be further investigated.
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Vitamin D är ett centralt hormonsystem för kalciumomsättningen, och adekvata nivåer är viktiga för skelettets hälsa. Ökad incidens av osteoporosrelaterade frakturer är ett globalt hälsoproblem, som inte bara medför lidande och ökad dödlighet, utan även ekonomiska problem för såväl individen som för samhället. Brist på vitamin D anses vara del av den patofysiologiska orsaken till osteoporos. Vitamin D-receptorn (VDR) har påvisats i ett stort antal mänskliga vävnader, och under de senaste decennierna har ökade bevis framkommit för att förutom effekter på skelettet, så reglerar vitamin D även många andra cellfunktioner. Exempelvis finns effekter på muskulaturen som skulle kunna påverka muskelstyrka och risk för fall.


Frågeställningar

I Att undersöka möjliga skillnader i vitamin D-nivåer mellan kvinnliga patienter av invandrar- och svenskt ursprung vid en svensk vårdcentral på breddgrad 60°N, där hälften av invånarna har invandrarbakgrund. En annan målsättning var att uppskatta skillnader mellan olika födoämnen med innehåll av vitamin D.

II Att identifiera vilka faktorer som påverkade vitamin D status hos svenska kvinnor på en vårdcentral.

III Att undersöka förhållandet mellan polymorfismer hos genen för CYP2R1 och nivåer av 25(OH)D3 och andra biokemiska parametrar i blodet (PTH, Ca, P och FGF23), bentäthet och frakturincidens.

IV Att undersöka samband mellan variationer hos genen för vitamin D-receptorn och mått för mätning av muskelstyrka, fysisk prestation och falltendens.

De viktigaste resultaten

I Signifikanta skillnader sågs mellan invandrade och svenska kvinnor beträffande cirkulerande vitamin D-nivåer i blodet och intag av vitamin D via födan. En positiv korrelation sågs mellan intaget av vitamin D via mat och nivåerna av 25(OH)D3.

II De faktorer som hade störst betydelse för vitamin D-nivåerna var genomgången solsemester senaste året och ursprung. Vitamin D-brist (plasma-25(OH)D3 <25 nmol/L) var vanlig hos kvinnor med invandrarbakgrund (61%). En invandrad kvinna och hälften av de svenskfödda kvinnorna hade nivåer av plasma-25(OH)D3 över 50 nmol/L.

III Signifikanta skillnader (4.6 – 18.5% skillnad mellan medelvärden för SNP-alleler) sågs för sex av åtta undersökta SNP:ar av genen för CYP2R1 och cirkulerande nivåer av 25-OH vitamin D, men inga korrelationer med nivåer av kalci um, fosfat, PTH eller FGF23 för någon av SNP:arna. Ett oväntat samband med bentäthet sågs, men inga skillnader i frakturincidens mellan varianter av polymorfismerna.

IV Genetiska variationer i VDR-receptorn förefaller inte att ha någon direkt effekt på muskelstyrka, fysisk prestation eller falltendens hos äldre svenska män.
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A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title “Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine”.)