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Vitamin D In Older Women - Fractures, Frailty and Mortality

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Vitamin D In Older Women

– Fractures, Frailty and Mortality

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CLINICAL AND MOLECULAR OSTEOPOROSIS RESEARCH UNIT | LUND UNIVERSITY



Vitamin D In Older Women

- Fractures, Frailty and Mortality

David Buchebner



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DOCTORAL DISSERTATION

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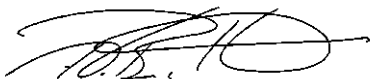
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Abstract <p>Vitamin D (25OHD) is essential for maintaining calcium homeostasis and inadequate levels have been associated with negative musculoskeletal as well as extraskelletal effects. Individuals at especially high risk of developing hypovitaminosis D are the elderly. The aim of this thesis was to investigate the association between 25OHD insufficiency (25OHD <50 nmol/L) and fractures, frailty and mortality. Additionally, we described the normal distribution of parathyroid hormone (PTH) in older women in relation to 25OHD and kidney function (eGFR) and investigated whether PTH was an independent predictor of frailty and mortality. Data was obtained from women participating in the Malmö Osteoporotic Risk Assessment Cohort (OPRA). This cohort consists of 1044 community-dwelling women, aged 75 years, who were followed prospectively for more than 15 years with reevaluations at ages 80 and 85 years. Blood biochemistry including 25OHD, PTH and eGFR was available at all time points. Information on fractures and mortality was continuously registered and a frailty index was constructed.</p> <p>Women with 25OHD levels <50 nmol/L, sustained between ages 75 and 80 years, had a higher 10-year risk of suffering a major osteoporotic fracture compared to women who maintained 25OHD levels ≥50 nmol/L (HR=1.8 [1.2-2.8], $p=0.008$). Mortality risk within 10 years of follow-up was significantly higher in 25OHD insufficient women compared to those with 25OHD > 75 nmol/L (75y: HR=1.4 [1.0-1.9], $p=0.04$ and 80y: HR=1.8, 95%CI=1.3-2.4, $p<0.001$). This increased risk remained after adjustment for smoking, diagnosis of osteoporosis and other comorbidities (at age 80). Between ages 75 and 80 years, PTH increased in 60% of all women (n=390) but increases of up to 50% above baseline values (64%; n=250) still resulted in PTH levels within the normal reference range (NRR), accompanied by lower 25OHD (74 vs 83 nmol/L, $p=0.001$). Only when increases were >50% was PTH elevated beyond the NRR (mean 7.1 ± 3.3 pmol/L). Here, a pronounced decline in eGFR (56 vs 61 mL/min/1.73 m², $p=0.002$) was found, despite no further decline in 25OHD. At age 85 years, half of the women had stable or decreased PTH levels (51%; n=169). PTH levels above NRR were not independently associated with mortality. At both ages 75 and 80, women with 25OHD <50 nmol/L were more frail compared to 25OHD sufficient women (0.23 vs 0.18; $p<0.001$ and 0.32 vs 0.25; $p=0.001$). Accelerated progression of frailty was not associated with lower 25OHD. Variables within the frailty-index that were associated with 25OHD, were those related to muscle strength and function. PTH was not independently associated with frailty</p> <p>In conclusion, 25OHD levels <50 nmol/L were associated with significant impairments of the musculoskeletal system (fractures, frailty) and predicted all-cause mortality in independently living older women. Parathyroid hormone was inversely correlated to 25OHD and eGFR but was not an independent predictor of frailty or mortality.</p>		
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Vitamin D In Older Women

- Fractures, Frailty and Mortality

David Buchebner



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Lund University, Faculty of Medicine
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Clinical and Molecular Osteoporosis Research Unit

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“Someday we'll look back on this and it will all seem funny”

Bruce Springsteen

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List of papers

This thesis is based on the following papers:

- I. Vitamin D Insufficiency over 5 Years Is Associated with Increased Fracture Risk - an Observational Cohort Study of Elderly Women
D. Buchebner, F. McGuigan, P. Gerdhem, J. Malm, M. Ridderstråle, K. Åkesson
Osteoporosis International 25: 2767, 2014
- II. Association Between Hypovitaminosis D in Elderly Women and Long- and Short-Term Mortality - Results from the Osteoporotic Prospective Risk Assessment Cohort
D. Buchebner, F. McGuigan, P. Gerdhem, M. Ridderstråle, K. Åkesson
Journal of the American Geriatrics Society 64:990–997, 2016
- III. Longitudinal Assessment of PTH in Community Dwelling Older Women - Elevations Are Not Associated with Mortality
D. Buchebner, L. Malmgren, A. Christensson, F. McGuigan, P. Gerdhem, M. Ridderstråle, K. Åkesson
Journal of the Endocrine Society, 2017:1(6); 615–624
- IV. Association Between Vitamin D and Frailty in Community-Dwelling Older Women
D. Buchebner, P. Bartosch, F. McGuigan, P. Gerdhem, M. Ridderstråle, K. Åkesson
In manuscript

Abbreviations

1,25OHD	1,25-dihydroxy-vitamin D3 or calcitriol (nmol/L)
25OHD	25-hydroxy-vitamin D3 or calcidiol (nmol/L)
95% CI	95% Confidence interval
AD	Anno domini (In the year of the Lord)
BMD	Bone mineral density (g/cm ²)
BMI	Body mass index (kg/m ²)
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration equation
Cr	Creatinine
CV	Coefficient of variation
CyC	Cystatine C
DEQAS	Vitamin D External Quality Assessment Scheme
DXA	Dual x-ray absorptiometry
eGFR	Estimated glomerular filtration rate (mL/min/1.73m ²)
FI	Frailty index
FRAX	Fracture Risk Assessment Tool
HPLC	High-performance liquid chromatography
HR	Hazard ratio
IDMS	Isotope dilution mass spectrometry
IOF	International Osteoporosis Foundation
IOM	Institute of Medicine
IU	International units
LC-MS	Liquid chromatography – mass spectrophotometry
NRR	Normal reference range
PTH	Parathyroid hormone (pmol/L)
SASP	Senescence-associated secretory phenotype
SD	Standard deviation
UVB	Ultraviolet B (light)
VDR	Vitamin D receptor
WHO	World Health Organization

Abstract

Vitamin D (25OHD) is essential for maintaining calcium homeostasis and inadequate levels have been associated with negative musculoskeletal as well as extraskeletal effects. Individuals at especially high risk of developing hypovitaminosis D are the elderly. The aim of this thesis was to investigate the association between 25OHD insufficiency (25OHD <50 nmol/L) and fractures, frailty and mortality. Additionally, we described the normal distribution of parathyroid hormone (PTH) in older women in relation to 25OHD and kidney function (eGFR) and investigated whether PTH was an independent predictor of frailty and mortality.

Data was obtained from women participating in the Malmö Osteoporotic Risk Assessment Cohort (OPRA). This cohort consists of 1044 community-dwelling women, aged 75 years, who were followed prospectively for more than 15 years with reevaluations at ages 80 and 85 years. Blood biochemistry including 25OHD, PTH and eGFR was available at all time points. Information on fractures and mortality was continuously registered and a frailty index was constructed.

Women with 25OHD levels <50 nmol/L, sustained between ages 75 and 80 years, had a higher 10-year risk of suffering a major osteoporotic fracture compared to women who maintained 25OHD levels \geq 50 nmol/L (HR=1.8 [1.2-2.8], $p=0.008$). Mortality risk within 10 years of follow-up was significantly higher in 25OHD insufficient women compared to those with 25OHD >75 nmol/L (75y: HR=1.4 [1.0-1.9], $p=0.04$ and 80y: HR=1.8, 95%CI=1.3-2.4, $p<0.001$). This increased risk remained after adjustment for smoking, diagnosis of osteoporosis and other comorbidities (at age 80). Between ages 75 and 80 years, PTH increased in 60% of all women (n=390) but increases of up to 50% above baseline values (64%; n=250) still resulted in PTH levels within the normal reference range (NRR), accompanied by lower 25OHD (74 vs 83 nmol/L, $p=0.001$). Only when increases were >50% was PTH elevated beyond the NRR. Here, a pronounced decline in kidney eGFR (56 vs 61 mL/min/1.73 m², $p=0.002$) was found, despite no further decline in 25OHD. At age 85 years, half of the women had stable or decreased PTH levels (51%; n=169). PTH levels above NRR were not independently associated with mortality. At both ages 75 and 80, women with 25OHD <50 nmol/L were more frail compared to 25OHD sufficient women (0.23 vs 0.18; $p<0.001$ and 0.32 vs 0.25; $p=0.001$). Accelerated progression of frailty was not associated with lower 25OHD. Variables within the frailty-index that were associated with 25OHD, were those related to muscle strength and function. PTH was not independently associated with frailty

In conclusion, 25OHD levels <50 nmol/L were associated with significant impairments of the musculoskeletal system (fractures, frailty) and predicted all-cause mortality in independently living older women. Parathyroid hormone was inversely correlated to 25OHD and eGFR but was not an independent predictor of frailty or mortality.

Introduction

The importance of sun-exposure in maintaining healthy bones and muscles has been known for a very long time. One of the earliest descriptions of rickets comes from Sorano of Ephesus, a Greek physician who practiced medicine in Alexandria and Rome during the first century AD. In his work “Gynecology”, he wrote *‘When the infant attempts to sit and to stand, one should help in its movements. For if it is eager to sit up too early and for too long a period it becomes hunchbacked (the spine bending because the little body has as yet no strength). If, moreover, it is too prone to stand up and desirous of walking, the legs may become distorted in the regions of the thighs’*¹.

The first clear description of rickets comes from Daniel Whistler who submitted a thesis for the degree of Doctor of Medicine in Leiden in 1645. He believed that rickets had an *‘antenatal origin due to the mother drinking too much alcohol’*².

During the 18th century, cod liver oil was successfully used to cure rickets and in 1822, Jędrzej Sniadecki, a Polish physician, emphasized the importance of sunlight to prevent and cure rickets³. However, it would take almost 100 years until sunlight exposure became an accepted cure of this disease⁴. In 1928, Adolf Windaus was awarded the Nobel prize in chemistry for his contribution in the discovery of vitamin D⁵.

Vitamin D and hormone D

Vitamin D is a fat-soluble vitamin synthesized in the skin during sunlight exposure or obtained from various nutritional sources such as fatty fish, mushrooms, eggs or dietary products fortified with vitamin D. Vitamin D₂ (ergocalciferol) is synthesized by UVB irradiation in plants or fungi whereas the main source for Vitamin D₃ (cholecalciferol) is its production in the skin during exposure to ultraviolet light (280-320 UVB)⁶.

In the liver, vitamin D₃ is then hydroxylated (25-hydroxylase) into 25-hydroxyvitamin D₃ (25OHD, calcidiol) which is the major circulating form of vitamin D. It is therefore considered the best indicator of vitamin D supply to the

body. Its half-life is two to three weeks and it has minimal biological potency. It is sometimes classified as *pre-hormone*.

A further hydroxylation (1α -hydroxylase) takes place in the kidneys resulting in 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃; calcitriol), the active metabolite of vitamin D. Calcitriol is a *secosteroid hormone* and its actions are mediated by the vitamin D receptor. It has a much shorter half-life of five to 15 hours.

Calcitriol is inactivated by 24-hydroxylase and metabolized into its water-soluble form, calcitroic acid, which is excreted through the bile and urine. Figure 1 gives a schematic overview of vitamin D metabolism.

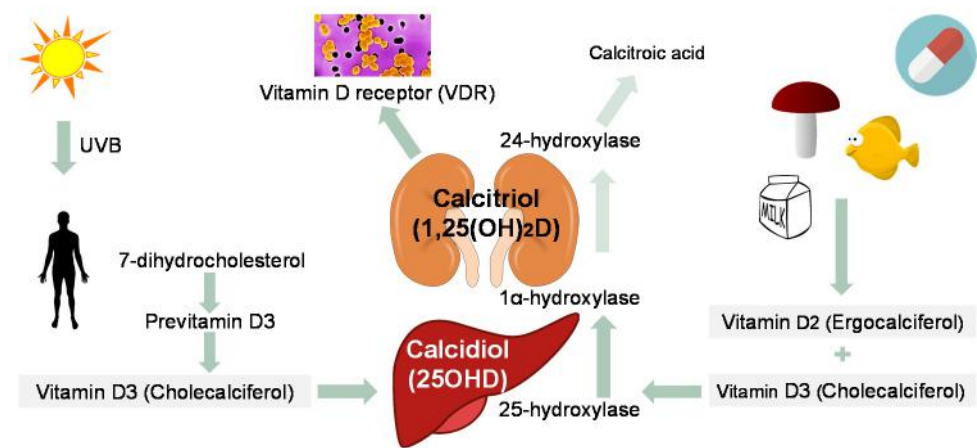


Figure 1. Vitamin D metabolism

Vitamin D is produced in the skin or obtained through dietary sources/supplements. It has to be activated in the liver and the kidneys and exerts its effects through interaction with vitamin D receptors in target tissues.

Vitamin D, together with parathyroid hormone (PTH) and calcitonin, is an important regulator of calcium homeostasis as shown in Figure 2.

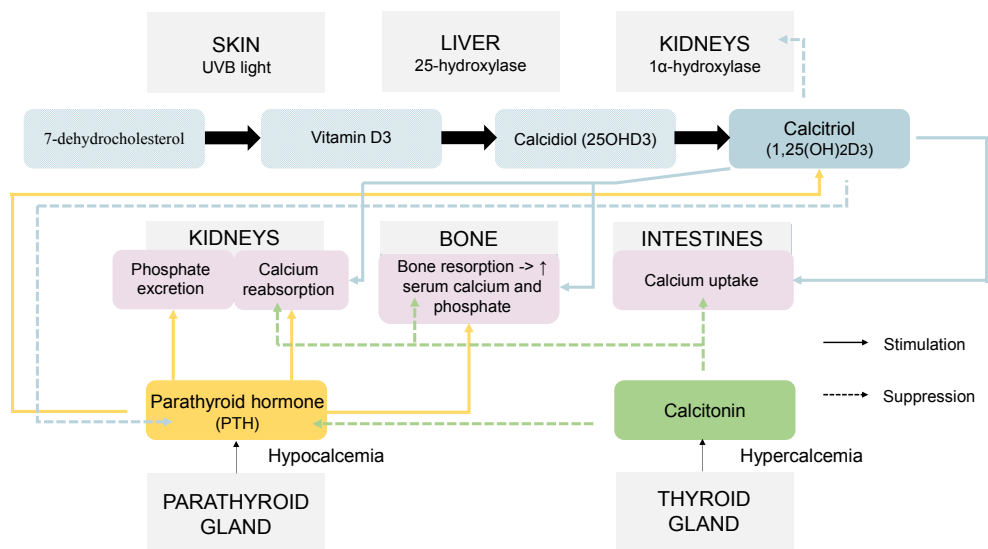


Figure 2. Calcium homeostasis

Calcium homeostasis is tightly regulated by parathyroid hormone, active vitamin D and calcitonin. The main organs regulating calcium homeostasis are the kidneys, bones and intestines.

Up-regulation of calcium is probably the most important, or at least the best described role of vitamin D. Most of the biological actions of vitamin D are initiated through interaction with the vitamin D receptor (VDR)⁷ which is present in almost all human cells^{8,9}. These findings give support for pleiotropic, also referred to as “non-classical”, effects that go beyond the musculoskeletal functions of vitamin D^{8,10}.

Most of the pleiotropic actions of active vitamin D are mediated through direct interaction and activation of nuclear vitamin D receptors initiating genomic activities that can vary from cell type to cell type⁸. Compared to these genomic, long-term actions of vitamin D, much faster, non-genomic, pathways have been identified which are mediated by membrane-bound receptors (mVDR)¹⁰. The stimulation of intestinal calcium absorption is an example of such a rapid, non-genomic action of vitamin D¹¹.

Epidemiology of hypovitaminosis D

Regardless of the definition of vitamin D insufficiency and deficiency, hypovitaminosis D is prevalent worldwide¹². In Europe, vitamin D concentrations below 25 nmol/L were found in 2-30% of adults¹³. Interestingly, in a study by Van der Wielen¹⁴ conducted in 11 European countries, mean vitamin D concentrations were higher in northern latitudes compared to the south despite the fact that ultraviolet radiation is more intense in southern European regions. Possible explanations might be found in different lifestyles (i.e. time spent outdoors, sun-seeking behavior), dietary intakes (i.e. oily fish, fortified dietary products) and different skin-types (grade of pigmentation).

The prevalence of hypovitaminosis D is much higher in geriatric patients and institutionalized individuals where inadequate vitamin D levels could be found in 75-90%^{12,15}. The most common risk factors for developing hypovitaminosis D are listed in Table 1.

Table 1. Risk factors for hypovitaminosis D

This table shows the most important risk factors for developing inadequate vitamin D levels

Person-related factors		External factors
Modifiable	Non-modifiable	
Insufficient dietary intake	Increasing age	Higher latitude
Low outdoor activity	Malabsorption	Lower altitude
Use of sunscreen	Skin pigmentation	More cloud cover
Use of covering clothes		Season of the year

Vitamin D, bone and muscle

Vitamin D is essential for maintaining healthy bones and muscles¹⁶ and can affect these tissues both directly (through regulation of proliferation, differentiation and apoptosis of bone and muscle cells) as well as indirectly (mainly through regulation of calcium absorption and PTH secretion). In bone tissue, vitamin D deficiency can cause osteomalacia and contribute to osteoporosis, two different diseases that will be described in more detail later (page 16 and 22-24). Inadequate vitamin D levels can also lead to impaired muscle function increasing the risk of falling. Ultimately, deteriorations in both bone and muscle tissues through pronounced and sustained hypovitaminosis D are involved in the pathogenesis of fractures. A simplified scheme showing the connection between vitamin D, falls and fractures is presented in Figure 3.

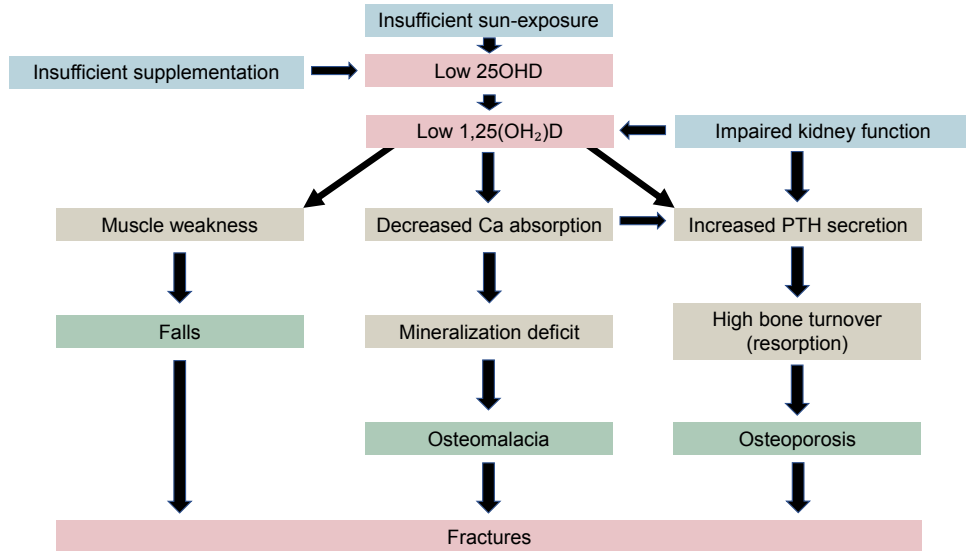


Figure 3. The influence of vitamin D on falls and fractures

Low vitamin D levels are linked to fractures through different pathways. An inadequate vitamin D status can lead to impaired muscle function which increases the risk of falling. Insufficient vitamin D levels also lead to decreased mineralization of the skeleton and increased PTH secretion stimulating bone turnover.

Actions of vitamin D in bone tissue

On the broadest level, the effects of active vitamin D on bone can be distinguished in a (1) general effect (non-genomic) enhancing calcium absorption from the gut, which is needed for mineralization of bone and (2) a more specific pathway (through genomic activation of the vitamin D receptor) regulating the activity of osteoblasts (bone forming cells) and osteoclasts (bone resorbing cells)¹⁷. Insufficient vitamin D levels also stimulate PTH secretion which increases bone turnover and, if prolonged, contributes to the development of osteoporosis¹⁸.

Actions of vitamin D in muscle tissue

Vitamin D also acts as an important regulator of calcium and phosphate homeostasis in muscle tissue influencing contractility, proliferation and differentiation of muscle cells. Analogous to the actions of vitamin D on bone tissue, these effects have been found to be mediated by vitamin D receptors (VDR) both through genomic (slow), and non-genomic (fast) pathways¹⁹.

As part of normal ageing, muscle strength and muscle function decline. In the elderly, the number of muscle fibers (mainly fast-twitch or type II fibers) is reduced

and the amount of fat and connective tissue is increased²⁰. Atrophy of type II muscle fibers has been found in muscle biopsies of vitamin D deficient adults and supplementation has been associated with an increase in the amount and size of muscle fibers²¹.

Osteomalacia

Severe vitamin D deficiency, usually below 12 nmol/L, leads to osteomalacia (in adults) or rickets (in children), a disease characterized by impaired mineralization of the skeleton²² and impaired muscle function²³. The main symptoms of osteomalacia include bone pain and proximal muscle weakness. In children, deformities of the skeleton are commonly seen. The insufficient amount of calcium available for mineralization leads to an accumulation of immature, unmineralized bone matrix (osteoids). This results in a skeleton that becomes softer and prone to deformation and fracture. In the diagnosis of osteomalacia, characteristic blood biochemistry with low vitamin D, calcium and phosphate and elevated PTH can be indicative and help distinguish between osteomalacia and osteoporosis. Bone x-rays may reveal typical cracks known as “Looser’s zones” (transverse translucent bands in the cortex of bones). Although rarely performed due to its invasive nature, the definitive diagnosis is based on iliac crest biopsy with double tetracycline labeling showing a reduced distance between tetracycline bands and an increase of osteoids (>10%).

Optimal vitamin D levels and dosage

25OHD levels below 25 nmol/L are commonly accepted as vitamin D *deficient*. We are however still lacking a consensus definition of vitamin D *insufficiency* as well as clear threshold values of what to regard as *optimal* vitamin D concentration. Production in the skin due to UVB radiation represents the major natural source of vitamin D^{24,25} and data from the Women’s Health Initiative study showed that vitamin D intake accounted for only 9% of the variance of 25OHD²⁶. However, in older people with limited sun exposure, dietary intake and supplementation are important determinants of the individuals’ vitamin D status^{26,27}.

Addressing this, in 2011, the American Institute of Medicine (IOM) published a report on dietary reference intakes for calcium and vitamin D²⁸. They concluded that a vitamin D intake of 600 IU per day for ages 1-70 years and 800 IU per day for ages 71 and above, corresponding to a serum vitamin D level of at least 50 nmol/L, would meet the requirements of at least 97.5% of the population. Higher levels were not consistently associated with any further benefit.

In contrast, the American Endocrine Society suggested higher supplementation doses (1500-2000 IU for ages 70 and above) targeting vitamin D levels above 75 nmol/L²⁹. These recommendations are, at least in part, based on the maximum suppression of parathyroid hormone seen with vitamin D levels above 75 nmol/L³⁰.

In a position statement by the International Osteoporosis Foundation (IOF)³¹, a dosage of 800-1000 IU per day was recommended in order to achieve prevention of fractures and falls in older adults. In individuals at high risk of vitamin D deficiency, daily doses up to 2000 IU might be needed in order to reach vitamin D levels of 75 nmol/L. The increase in serum 25OHD can be estimated to be about 2.5 nmol/L per 100 IU.

In Sweden, the Swedish Osteoporosis Society, suggests to define vitamin D deficiency as levels below 25 nmol/L, insufficiency between 25 and 50 nmol/L and sufficiency above 50 nmol/L³².

It has to be noted that all of these recommendations are primarily based on data available for the prevention of falls and fractures. It is therefore unclear whether the same thresholds and dosage recommendations are applicable to the effects of vitamin D outside the musculoskeletal system.

Functions of parathyroid hormone

Parathyroid hormone (PTH) is a polypeptide secreted by the parathyroid glands. The major targets of PTH are the kidneys, skeleton and intestines. As shown in Figure 2, PTH plays an important role in regulating calcium-phosphate homeostasis. Hypersecretion of PTH can be seen in either primary or secondary hyperparathyroidism.

Primary hyperparathyroidism is caused by adenomas or hyperplasia of the parathyroid glands. Patients with hyperparathyroidism are mostly asymptomatic but a constant elevation of PTH increases bone remodeling³³ (both resorption and formation) and can lead to a marked elevation of serum calcium as well as renal involvement (nephrolithiasis and nephrocalcinosis). If prolonged, hyperparathyroidism results in bone resorption mainly of cortical bone and is characterized by bone pain, proximal muscle weakness and pathological fractures³⁴. Neuropsychiatric symptoms can also occur including anxiety, depression, confusion, memory loss, irritability, difficulty in concentration and sleep disorders³⁵. From a bone perspective, these conditions can be relevant due to an increase in the risk of falls.

Secondary hyperparathyroidism is caused by diseases outside the parathyroid glands, the two major causes being impaired kidney function with a consecutive

impairment of the production of active vitamin D or an insufficient vitamin D status per se. The symptoms of patients with secondary hyperparathyroidism are similar to those of individuals suffering from primary hyperparathyroidism with the exception of vitamin D related symptoms being more prominent (i.e. muscle weakness). Hypercalcemia and hyperphosphatemia might eventually be seen in severe cases driven by calcium and phosphate efflux from the skeleton³⁶.

Vitamin D and ageing

As previously stated, vitamin D is important for calcium- and phosphate homeostasis. Therefore, vitamin D plays an essential role in maintaining bone health as we get older. There are two major reasons for hypovitaminosis D being common in older people. Firstly, the lack of substrate (from inadequate sun-exposure and nutritional intake) is common in older adults. Secondly, the ability to produce vitamin D in the skin is reduced and so is the formation of active vitamin D in response to PTH secretion in the aging kidneys³⁷. Being the main regulator of calcium absorption, decreased bioavailability of active vitamin D leads to decreased calcium absorption.

In the elderly, apart from bone and muscle related consequences, vitamin D may play a role in pathways related to healthy ageing including cell differentiation, proliferation and cellular communication³⁸⁻⁴⁰. Vitamin D also has anti-angiogenic effects⁴¹, can control apoptosis in deteriorated cells involved in the process of ageing⁴² and carries out anti-oxidative actions⁴³. Vitamin D receptors are expressed by cells involved in the immune system (i.e T-cells, antigen-presenting cells, thymocytes) and vitamin D was found to play an important role in the up- and down-regulation of the immune response⁴⁴⁻⁴⁶. Finally, vitamin D may have neuroprotective effects by promoting neuronal cell survival^{47,48}.

Vitamin D and the senescence of cells

An area of increasing interest is to understand the importance of senescence during ageing. Accumulation of DNA damage and other cellular stressors can cause senescence, a process in which cells cease dividing and undergo distinctive phenotypic alterations (SASP)⁴⁹. Turning “normal” cells into senescent cells is important in order to protect against cancer but also seems to play a role in normal cell development, tissue repair and ageing. In a recent study by Farr et al⁵⁰ which investigated the role of senescent cells in age-related bone loss in older mice, “clearing” these cells (through senolytic drugs, by inducing “suicide” genes or inhibiting SASP) resulted in a significant increase of bone mass. Although in its

early stages, further research could possibly open a new field of treatments for a variety of age-related disorders.

In vitamin D receptor-knockout mice, the lack of vitamin D signaling was found to cause premature senescence in vascular smooth muscle cells, mediated by increased angiotensin II⁵¹ and at least in vitro, vitamin D has been shown to protect endothelial cells from irradiation-induced senescence and apoptosis⁵².

Bone cells, bone remodeling and bone loss

The human skeleton is made up of two types of bones.

Cortical, or lamellar bone, makes up 80 percent of the skeleton and covers the outer shell (cortex) of most bones as well as the shaft (diaphysis) of long bones with its main function being protection. It consists of packed osteons and is characterized by slow bone turnover.

Cancellous, or trabecular bone, is mainly found at the end of long bones (epiphysis), proximal to joints and within the interior of vertebrae. It has a much lower density making it softer and more flexible. Trabecular bone consists of plates (trabeculae) and bars surrounding irregular cavities giving place to red bone marrow. It is highly vascular and shows a high metabolic activity and higher bone turnover.

Figure 4 illustrates normal lifetime changes in bone mass.

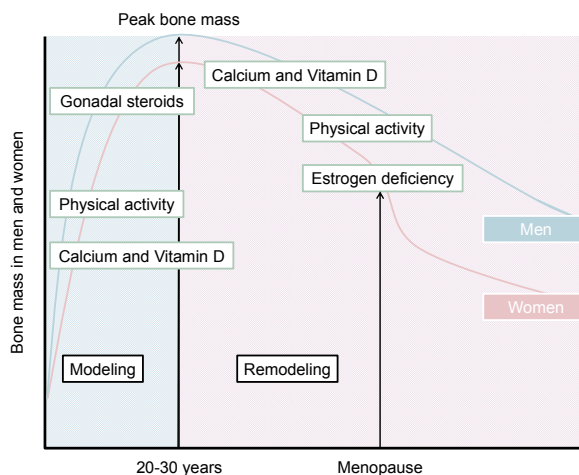


Figure 4. Bone mass throughout life in men and women

During childhood and adolescence, bones are sculptured by modeling. Peak bone mass is usually reached between ages 20 and 30 years. Constant bone remodeling in adults leads to bone loss that ranges from 0,5-1% per year. A pronounced loss of bone is seen in women in the first postmenopausal years due to estrogen deficiency.

Bone tissue undergoes constant modeling and remodeling throughout life. It is carried out by bone-forming cells (osteoblasts) and bone-resorbing cells (osteoclasts)⁵³. During childhood and adolescence, bones are sculptured by modeling, which is essential for growth. During modeling, osteoblasts and osteoclasts work more or less independently allowing for bone resorption at one site and bone formation at another (in contrast to remodeling).

The maximum bone mass (peak bone mass) is usually reached between 20 and 30 years of age. From here on, bone remodeling, a process of close interaction between osteoblasts and osteoclasts, is predominant⁵⁴.

In the bone remodeling process, osteocytes act as “commander and control” cells leading to activation of resting osteoblasts on the bone surface and marrow stromal cells in response to micro-damage. Activation of these cells initiates the process of differentiation towards fully-differentiated osteoblasts. At the same time, these progenitors also stimulate the differentiation of osteoclast precursors. Osteoclasts may then reinforce activation of osteoblast progenitors. A simplified scheme of the bone remodeling cycle is shown in Figure 5.

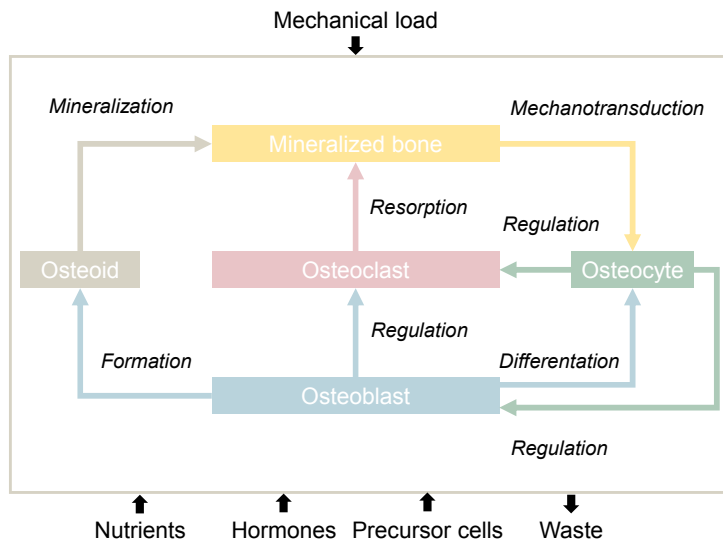


Figure 5. Bone remodeling cycle

Bone tissue undergoes constant remodeling which is carried out by osteoblasts and osteoclasts. Imbalance in this process, either caused by increased bone resorption or decreased bone formation, can lead to osteoporosis

The process of bone resorption takes approximately two weeks while the time frame for bone formation is up to three months. Termination of the bone remodeling cycle is once again induced by osteocytes. Bone remodeling is controlled by various local and systemic factors as shown in Tables 2 and 3.

Table 2. Regulating factors of bone resorption

Stimulation	Inhibition
Rank Ligand (RANKL): expressed by osteoblasts, binds to RANK receptor on osteoclast precursors	Osteoprotegerin (OPG): acts as decoy to RANK receptor, inhibiting interaction between RANKL and RANK receptor
Interleukins 1 and 6 (IL-1, IL-6): increase RANKL expression	Interleukins 4, 10, 13 and 18: inhibit osteoclast function
Tumor necrosis factor α (TNF- α): increases RANKL expression	Interferon γ : inhibits osteoclast function
Prostaglandin E2 (PGE2): increases RANKL expression	Transforming growth factor β (TGF β): increases OPG by osteoblasts and stromal cells and increases osteoclast apoptosis
Macrophage-colony-stimulating factor-1 (CSF-1): induces osteoclast differentiation	Calcitonin: inhibits osteoclast activity, leads to immobility and reduces the osteoclastogenic effect of RANKL
1,25 Dihydroxyvitamin D ₃ : stimulates osteoclastogenesis via increased RANKL expression on osteoblasts	Sex steroids (Estrogen and Testosterone): downregulate RANKL, upregulate OPG and TGF- β
Parathyroid hormone (PTH): increases RANKL expression	
Thyroid hormone: increases expression of RANKL, IL-6 and PGE2	
Cortisol: increases expression of RANKL and CSF-1; decreases expression of OPG	

Table 3. Regulating factors of bone formation

Stimulation	Inhibition
Transforming growth factor β (TGF- β): induces differentiation and proliferation of osteoblasts	Sclerostin (secreted by osteocytes): inhibits Wnt-LRP5 mediated bone formation
Fibroblast growth factors (FGF-2): stimulates proliferation of osteoblasts	Interleukins 1 β and 7: inhibit cell replication and protein synthesis in osteoblasts
Insulin-like growth factor-1 (IGF-1): controls proliferation and differentiation of osteoblasts	Interferon γ : inhibits osteoblast function
Prostaglandin E2 (PGE2): stimulates bone formation and fracture healing	Tumor necrosis factor α (TNF- α): inhibits DNA and collagen synthesis in osteoblasts
Wnt coreceptor low-density lipoprotein receptor-related protein 5 (Wnt-LRP5): prevents apoptosis and stimulates replication of osteoblasts, decreases serotonin excretion in intestines	Cortisol: delays and prevents cell differentiation and induces apoptosis of osteoblasts
1,25 Dihydroxyvitamin D ₃ : increases osteoblast proliferation and differentiation	1,25 Dihydroxyvitamin D ₃ : induces osteoblast apoptosis
Parathyroid hormone (PTH): increases osteoblast proliferation and differentiation, enhances the Wnt- pathway, inhibits sclerostin and induces synthesis of IGF-1	Serotonin (produced in the intestines): inhibits differentiation of osteoblasts
Growth hormone (GH): stimulates osteoblast proliferation directly and also via IGF-1	
Leptin (excreted by the hypothalamus): stimulates the differentiation of stromal cells to osteoblasts, inhibits differentiation into adipocytes	
Serotonin (produced in the brain): stimulates osteoblast differentiation	

Bone loss during adult life ranges between 0.5-1% per year⁵⁵ but estrogen deficiency during menopause leads to an increase in bone loss, mostly within the first 8-10 postmenopausal years. Although both bone resorption and formation increase, the latter is to a minor extent, leading to rates of bone loss around 2-4% annually⁵⁶. Glucocorticoid treatment can have an even faster and more profound impact on bone loss with estimated reductions in bone density of up to 20%⁵⁷.

Osteoporosis

Definition

Osteoporosis is a multifactorial disorder of the bone remodeling cycle leading to an imbalance between bone formation and bone resorption. It is a systemic skeletal disease characterized by low bone mass and microarchitectural deterioration leading to increased fragility and susceptibility to fracture⁵⁸.

In contrast to *osteomalacia* (described on page 18 in more detail), which is characterized by impaired mineralization of bones (“*too little calcium in bone*”), *osteoporosis* leads to a decrease in bone mass and altered microarchitecture. The mineral-matrix ratio is usually intact (“*Too little bone*”). Picture 1 shows a comparison between normal bone and osteoporotic bone.



Picture 1. Normal and osteoporotic bone

This picture shows the structure of normal bone compared to osteoporotic bone. Osteoporosis is characterized by decreased bone mass in otherwise normally mineralized bones.

Adapted from Osteoporosis. (2017, September 24). In Wikipedia, The Free Encyclopedia., from <https://en.wikipedia.org/w/index.php?title=Osteoporosis&oldid=802147257>

Epidemiology

In 2013, twenty-two million women and more than five million men were estimated to suffer from osteoporosis in the European Union⁵⁹. Worldwide, 30-50% of all women and 15-30% of all men will suffer a fracture related to osteoporosis above the age of 50⁶⁰.

Classification and major causes

Osteoporosis can be classified as primary (caused by age related loss of bone or estrogen deficiency), or secondary (resulting from other diseases or medical conditions). The predisposition to osteoporosis is influenced by both environmental and lifestyle factors such as body weight, physical activity, smoking, alcohol use and diet as well as genetic factors⁶¹⁻⁶³. The most common causes of osteoporosis are shown in Table 4.

Table 4. Common causes of osteoporosis

Primary osteoporosis is the most common form of osteoporosis. 5-20% of all postmenopausal women are affected by osteoporosis. Glucocorticoid treatment is the most common cause of secondary osteoporosis.

Primary osteoporosis
Postmenopausal osteoporosis: Estrogen deficiency
Senile osteoporosis: decreased bone formation, vitamin D insufficiency and prolonged elevation of PTH, decreased calcium absorption
Secondary osteoporosis
Glucocorticoids: primarily suppression of bone formation; stimulation of osteoclastogenesis, decreased calcium absorption
Aromatase inhibitors: increased bone turnover
Hyperthyroidism: increased bone turnover due to stimulation by thyroid hormones and loss of inhibition by TSH
Androgen deficiency: Decreased bone formation, decreased aromatization to estrogen
Hyperparathyroidism: increased bone turnover, increased RANKL-expression of osteoblasts
Malabsorption and inflammatory bowel disease: decreased calcium absorption and secondary hyperparathyroidism, proinflammatory cytokines enhance osteoclastogenesis
Chronic kidney disease: abnormalities of calcium, phosphate, parathyroid hormone and vitamin D
Reumatoid arthritis: proinflammatory cytokines increase osteoclast activity, sclerostin inhibits bone formation
Drugs: <i>Anticonvulsants</i> (accelerated vitamin D metabolism), <i>Anti-retroviral therapy</i> (increased RANKL-expression), <i>Heparin</i> (inhibition of osteoblast differentiation, binding to OPG allows RANKL to induce osteoclastogenesis), <i>Loop diuretics</i> (inhibition of calcium absorption and increase in renal excretion)

Diagnosis

The diagnosis of osteoporosis is based on a measurement of bone mineral density (BMD) assessed by dual-energy X-ray absorptiometry (DXA) which calculates the bone mass per unit area (g/cm^2)⁶⁴.



Picture 2. DXA machine and radiology nurse at Hallands Hospital Halmstad
Photograph by David Buchebner

BMD measurements are usually performed at the lumbar spine and femoral neck and presented as T-scores. The T-score describes the number of standard deviations by which the individuals' BMD differs from the mean value expected in a young reference population. A T-score at -2.5 or below is defined as osteoporosis⁶⁵. In older patients however, degenerative changes in the lumbar spine can make for a less certain assessment of osteoporosis measured at this site⁶⁶.

A limitation of DXA is that it cannot distinguish between low bone mineral density caused by osteoporosis and osteomalacia. For the latter, laboratory findings such as low vitamin D levels, elevated PTH and low calcium are often indicative.

Osteoporotic Fractures

Definition

The clinical outcome of untreated or insufficiently treated osteoporosis is fracture. Classical osteoporotic fractures are those of the hip, vertebrae and wrist and are per definition fractures caused by *low-energy trauma*. They are often defined as fractures occurring when *falling* from the same level. Kanis et al defined osteoporotic fractures as occurring at a site associated with *low BMD* and which at the same time increased in incidence after the age of 50⁶⁷.

The underlying pathology of osteoporotic fractures, also called fragility fractures, is a decreased bone mass and quality which alters the resistance of bone to mechanical load. Fractures of the distal forearm and the hip are typically caused by falls, but the height and direction of falls and the individual's ability or disability to react to falling vary between these two fracture types⁶⁸.

Types of fracture and epidemiology

Fractures of the distal forearm are typically the first clinical fractures before the age of 75 and are almost exclusively caused by falls⁶⁹, thus the distinction between low-energy and high-energy fractures is not always clear. The direction of falling is usually forwards or backwards⁶⁸.

Hip fractures, the most severe of the osteoporotic fractures, typically occur in older and frailer individuals and are usually caused by falling sideways⁶⁸. The mean age of patients suffering from a hip fracture in Sweden is 80 years for women and 76 years for men. Given the higher age of hip fracture patients, impaired muscle function, postural instability and medication affecting balance are common factors contributing to fracture risk^{70,71}. These fractures are associated with high morbidity and mortality⁷² and lead to a high socioeconomic burden.

The incidence of vertebral fractures is similar in middle-aged men and women but increases significantly for women during the early post-menopausal years. In contrast to fractures of the radius and hip, vertebral fractures often occur without trauma and can be asymptomatic. The prevalence of vertebral deformities increases with age⁷³ and is estimated to around 20% in postmenopausal women with increases up to above 60% in the oldest old^{73,74}. However, only a third of all vertebral fractures in women and less than 50% in men are clinically diagnosed^{75,76}.

In Sweden, the lifetime risk for an osteoporotic fracture at age 50 years is 46% for women and 22% for men which is among the highest in the world⁷⁷. The exact

reason for this is unclear. Higher incidence rates have been reported during winter compared to summer months and fracture risk seems to be higher in Northern Sweden compared to the South indicating that the amount of UV-exposure and thus amount of available vitamin D might play a role⁷⁸. Lifestyle and life expectancy could be contributing factors. Interestingly, fracture risk seems to be lower in immigrants compared to Swedish-born individuals, even 40 years post immigration which could indicate a genetic predisposition for fractures⁷⁹. Incidence rates of osteoporotic fractures in Sweden are illustrated in Figure 6.

Worldwide, nearly nine million fractures are caused by osteoporosis annually which translates to about 1000 fractures per hour. One third of these fractures occur in Europe⁵⁹.

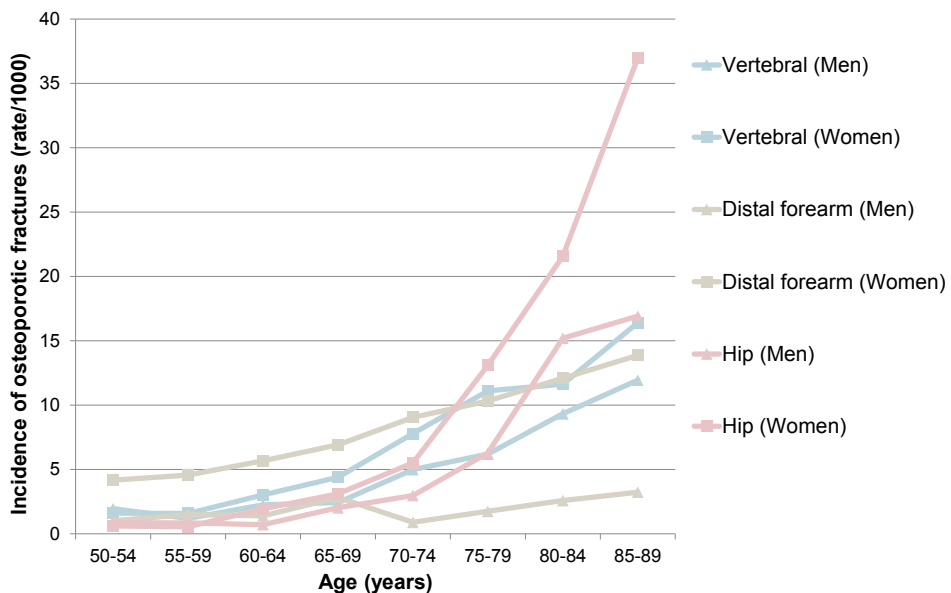


Figure 6. Incidence of osteoporotic fractures in Sweden
Adapted from Kanis et al.⁸⁰. The figure illustrates the incidence of fractures occurring at the hip, vertebrae and distal forearm in men and women between ages 50 and 89.

Risk factors

The risk of sustaining a fracture is correlated to bone mineral density and approximately doubles for each standard deviation decrease in BMD⁸¹. However, the majority of fragility fractures occurs in patients classified as osteopenic rather than osteoporotic⁸². Hence, several other risk factors, apart from low BMD, have to be considered when assessing fracture risk. Advanced age is the single most important risk factor. Between ages 50 and 80, the risk of fracture increases 30-fold,

independently of BMD⁸¹. Fractures are twice as common in women as in men and a family history of hip fracture in parents has been associated with a 2-fold increase in the risk of this type of fracture⁸³. A previous fracture is associated with a 2- to 4-fold increase in the risk of a subsequent fracture⁷⁷ and patients sustaining fragility fractures are more likely to have a history of falls⁸⁴. As mentioned previously, hip fractures and fractures of the distal forearm are almost exclusively caused by falling. The most relevant risk factors for fragility fractures are summarized in Table 5.

Table 5. Risk factors for fragility fractures

Age	The incidence of fragility fractures increases with age; most vertebral fractures occur around age 70y; the peak of hip fractures is reached around 80y
Gender	Fragility fractures are twice as common in women as in men
Low bone mineral density (BMD)	Every 1 standard deviation reduction in BMD equates approximately to a doubling of the relative risk of fracture.
Parental history of hip fracture	Indicator of genetic risk of fragility fracture
Previous fracture	2-4 fold increase in fracture risk
Falls	Fragility fractures usually occur during falling
Hormones	Premature menopause (< age 45y); androgen deprivation therapy
Medical conditions associated with bone loss	Rheumatoid arthritis, inflammatory bowel disease (e.g. Crohn's disease, ulcerative colitis), malabsorption (e.g. coeliac disease, pancreatic insufficiency), cystic fibrosis, hyperthyroidism, hyperparathyroidism, vitamin D insufficiency, immobilization, chronic obstructive pulmonary disease, diabetes mellitus type 1 and chronic renal and hepatic disease.
Drugs associated with bone loss	Corticosteroids, aromatase inhibitors, androgen deprivation therapy, some anti-epileptic medications and glitazones.
Lifestyle factors	Smoking and alcohol intake \geq 3 units per day

The risk of sustaining a low-energy fracture can be estimated using the Fracture Risk Assessment Tool, an online calculator developed by the University of Sheffield in association with the World Health Organization (FRAX®; <https://www.sheffield.ac.uk/FRAX/tool.aspx?lang=en>). Picture 3 shows the result of a FRAX® calculation.

Calculation Tool

Please answer the questions below to calculate the ten year probability of fracture with BMD.

Country: **UK** Name/ID:

Questionnaire:

1. Age (between 40 and 90 years) or Date of Birth
 Age: Date of Birth: Y: M: D:

2. Sex Male Female

3. Weight (kg)

4. Height (cm)

5. Previous Fracture No Yes

6. Parent Fractured Hip No Yes

7. Current Smoking No Yes

8. Glucocorticoids No Yes

9. Rheumatoid arthritis No Yes

10. Secondary osteoporosis No Yes

11. Alcohol 3 or more units/day No Yes

12. Femoral neck BMD (g/cm²)
 Select BMD

BMI: 23.9
The ten year probability of Fracture (%)

without BMD	
Major osteoporotic	38
Hip Fracture	26
View NOGG Guidance	

Risk factors

For the clinical risk factors a yes or no response is asked for. If the field is left blank, then a "no" response is assumed. See also notes on risk factors.

The risk factors used are the following:

Age	The model accepts ages between 40 and 90 years. If ages below or above are entered, the programme will compute probabilities at 40 and 90 year, respectively.
Sex	Male or female. Enter as appropriate.
Weight	This should be entered in kg.
Height	This should be entered in cm.
Previous fracture	A previous fracture denotes more accurately a previous fracture in adult life occurring spontaneously, or a fracture arising from trauma which, in a healthy individual, would not have resulted in a fracture. Enter yes or no (see also notes on risk factors).
Parent fractured hip	This enquires for a history of hip fracture in the patient's mother or father. Enter yes or no.
Current smoking	Enter yes or no depending on whether the patient currently smokes tobacco (see also notes on risk factors).
Glucocorticoids	Enter yes if the patient is currently exposed to oral glucocorticoids or has been exposed to oral glucocorticoids for more than 3 months at a dose of prednisolone of 5mg daily or more (or equivalent doses of other glucocorticoids) (see also notes on risk factors).
Rheumatoid arthritis	Enter yes where the patient has a confirmed diagnosis of rheumatoid arthritis. Otherwise enter no (see also notes on risk factors).
Secondary osteoporosis	Enter yes if the patient has a disorder strongly associated with osteoporosis. These include type I (insulin dependent) diabetes, osteogenesis imperfecta in adults, untreated long-standing hyperthyroidism, hypogonadism or premature menopause (<45 years), chronic malnutrition, or malabsorption and chronic liver disease
Alcohol 3 or more units/day	Enter yes if the patient takes 3 or more units of alcohol daily. A unit of alcohol varies slightly in different countries from 8-10g of alcohol. This is equivalent to a standard glass of beer (285ml), a single measure of spirits (30ml), a medium-sized glass of wine (120ml), or 1 measure of an aperitif (60ml) (see also notes on risk factors).
Bone mineral density (BMD)	(BMD) Please select the make of DXA scanning equipment used and then enter the actual femoral neck BMD (in g/cm ²). Alternatively, enter the T-score based on the NHANES III female reference data. In patients without a BMD test, the field should be left blank (see also notes on risk factors) (provided by Oregon Osteoporosis Center).

Picture 3. FRAX®

FRAX helps identifying patients at high risk of osteoporotic fractures. Several clinical risk factors for fractures are included in the model, which can be used with or without DXA-scanning. The result is presented as a 10-year fracture risk. According to the Swedish National Board of Health and Welfare, a 10-year probability of major osteoporotic fractures $\geq 15\%$ should be considered a high risk of fracture.

<http://www.socialstyrelsen.se/nationellarkiktlinjerforforelseorganenssjukdomar/sokiriktlinjerna/videnfrakturriskenligfraxutan> Picture derived from the online FRAX calculator at <https://www.sheffield.ac.uk/FRAX/>

In the FRAX® model, the risk of hip and major osteoporotic fractures is calculated in men or women based on age, body mass index (BMI) and independent risk variables including prior fragility fracture, parental history of hip fracture, current tobacco smoking, ever long-term use of oral glucocorticoids, rheumatoid arthritis, other causes of secondary osteoporosis and daily alcohol consumption of 3 or more units daily.

It can be used with or without BMD (T-score) derived from a DXA measurement. While FRAX® helps in identifying individuals at high risk of fracture, it doesn't consider falls and vitamin D levels, two important risk factors for fractures in older adults.

Vitamin D and fractures

The association between low vitamin D levels and fracture risk, in particular the risk of hip fractures, has been shown repeatedly⁸⁵⁻⁸⁹. This is also true for the association between vitamin D and muscle function^{19,90}. However, questions remain whether the risk of fractures can be lowered by reversing vitamin D insufficiency or deficiency. Results from randomized controlled trials testing the effects of vitamin D supplementation are inconsistent⁹¹⁻⁹⁷. Table 9 in the appendix section (pages 74-76) shows a summary of the most important randomized controlled trials investigating the fracture preventive effect of vitamin D supplementation.

The following limitations of interventional studies may be of importance for this discrepancy seen between observational data and randomized controlled trials. Insufficient dosage, low adherence to supplementation and short treatment periods may have influenced the outcome. Moreover, 25OHD was not routinely assessed in all patients, and if so, most studies relied on single measurements at baseline, hence, missing information on whether participants reached sufficient vitamin D levels. In most studies, vitamin D supplementation was administered in conjunction with calcium which makes it difficult to interpret the effects of vitamin D solely.

A dosage of at least 800 IU per day seems to be required in order to achieve a significant risk reduction of fractures. The aspect of age seems to be important since the fracture- and fall-preventive effect of vitamin D appears to be highest in elderly, institutionalized individuals⁹⁴. Whether this can be explained through the impact of age per se or the fact that older individuals presumably have lower vitamin D levels compared to a younger population is debatable.

Effects of vitamin D outside the musculoskeletal system

Vitamin D receptors (VDR) are almost ubiquitously present in the human body¹⁰ and, at least in theory, the actions of vitamin D should extend beyond the “classical” effects on the musculoskeletal system. In recent years, there has been a profound interest in research trying to explore possible extraskeletal mechanisms of vitamin D and data from epidemiological research has repeatedly shown that low vitamin D levels are associated with various negative health outcomes. To name a few, associations have been found between low vitamin D and the risk of developing *colorectal cancer*⁹⁸⁻¹⁰², *breast cancer*¹⁰³⁻¹⁰⁵ and *prostate cancer*^{106,107}. Moreover, inadequate vitamin D levels have also been associated with poorer cancer-survival¹⁰⁸⁻¹¹⁰. In population studies, vitamin D deficiency has also been associated with increased risk of *cardiovascular disease*¹¹¹⁻¹¹³, *type-II diabetes*^{114,115} and autoimmune diseases such as *multiple sclerosis*¹¹⁶, *type-I diabetes*¹¹⁷ and *rheumatoid arthritis*¹¹⁸.

Vitamin D and mortality

Fracture-related mortality

Vitamin D insufficiency is common in hip fracture patients¹¹⁹ and has been associated with poor functional recovery¹²⁰. Hip fractures in older individuals are associated with increased mortality in the short term¹²¹ which could probably be explained by fracture related complications such as infections and cardiovascular diseases⁷². However, data from epidemiological studies suggests that the elevated risk of dying persists for up to 10 years after a low-trauma fracture¹²². A possible explanation could be the progressive functional decline following a fracture, ultimately leading to a state of increased frailty and disability. Although vitamin D supplementation has been shown to decrease post-fracture mortality¹²³, controversy remains over the causal relationship between inadequate vitamin D levels and mortality¹²⁴

All-cause and disease-specific mortality

In recent years, increasing evidence has emerged showing relations between low vitamin D levels and cause-specific deaths due to cardiovascular disease and cancer as well as all-cause mortality^{125,126}. However, our knowledge is limited regarding the optimal vitamin D concentration needed in older adults in order to lower the risk of dying. Moreover, chronic hypovitaminosis D in the oldest old and its association to mortality has not been sufficiently investigated

What do we know from randomized controlled trials?

Results from randomized controlled trials are inconsistent¹²⁷ and in most cases, these trials have failed to provide evidence supporting the findings of observational studies. At least to some extent, this apparent discrepancy may be explained by several limitations of randomized controlled trials conducted so far. For instance, in a sub-study of the Women's Health Initiative, supplementation of more than 18 000 women with vitamin D and calcium did not decrease the risk of colorectal cancer¹²⁸. However, women were supplemented with only 400 IU per day, a dosage that is unable to substantially increase 25OHD levels and adherence to supplementation was rather low (~60%)¹²⁹. In the RECORD-trial¹³⁰, a non-significant trend towards decreased disease mortality and cancer mortality was found in participants supplemented with 800 IU per day. Once again, compliance to supplementation was rather poor (~60%)¹³¹. 25OHD levels were only measured in about 1% of participants at baseline and after 1 year of supplementation. Mean 25OHD levels increased from 38 nmol/L to 62 nmol/L which could be regarded sub-optimal. The negative results from a trial by Trivedi et al¹³², using 100 000 IU every four months, could have been influenced by the small sample size and short follow-up period. In a trial conducted in Nebraska, USA¹³³, 1100 IU per day were associated with a significant reduction of various cancer types, however, the number of participants developing cancer was very low and the results should therefore be interpreted cautiously. None of these studies was designed with mortality as primary outcome.

Several major randomized controlled trials, like the Vitamin D and Omega-3 trial (VITAL)¹³⁴ (2000 IU/d, cardiovascular disease and cancer), the D-Health study¹³⁵ (60000 IU/m, total mortality and cancer), the Vitamin D and Longevity (VIDAL) trial (<http://vidal.lshtm.ac.uk>) (100000 IU/m, total mortality and cancer) or the Finnish Vitamin D Trial (FIND) (1600 IU/d or 3200 IU/d, cardiovascular disease and cancer) are currently ongoing and first results are expected within the near future.

Frailty

What is frailty?

Ageing is inevitably linked to a gradual decline in physical functioning. However, the pace of decline varies among individuals of similar chronological age. While some appear to be robust and resilient, others show a more rapid loss of physical functions leading to vulnerability for adverse health outcomes¹³⁶. This accelerated decline, which is different from the one expected as part of healthy ageing, is

commonly described by the term “frailty”. Frailty increases the risk for falls, fractures, disability, comorbidity, health care expenditure and premature mortality¹³⁷. Despite the lack of a single operational definition of frailty, consensus has been reached on the following characteristics: Frailty (1) is a clinical syndrome (2) that increases vulnerability and maladaptive response to stressors (3) and might be reversible with interventions¹³⁸.

Epidemiology

The prevalence of frailty in community-dwelling individuals is estimated to approximately four percent between ages 65 and 74 climbing up to 25% above the age of 85¹³⁹. In Europe, higher prevalence rates are seen in southern European countries compared to the north¹⁴⁰.

Etiology and pathogenesis

Not much is known about the underlying mechanisms and exact pathogenesis of frailty. However, the etiology seems to be multifactorial as illustrated in Figure 7. Genetic predisposition, metabolic factors, lifestyle and environmental stressors as well as acute and chronic diseases are seen as potential etiologic factors. Chronic inflammation and activation of the immune system might play an integral role in its pathogenesis¹⁴¹⁻¹⁴³.

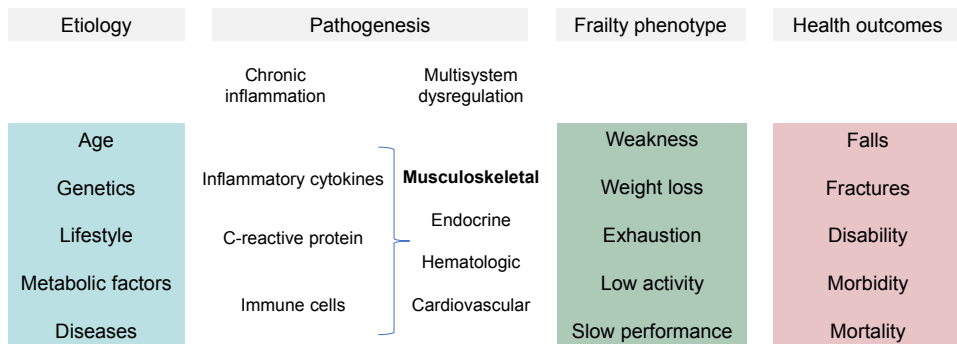


Figure 7. Potential etiology and pathogenesis of frailty

This figure illustrates the hypothesized, multifactorial pathway in the development of frailty.

nor is the frailty-predictive value of vitamin D insufficiency. A sufficient vitamin D status might be important for healthy ageing¹⁶⁰ but whether vitamin D supplementation is able to prevent or improve frailty in the oldest old is unclear.

Measurement of frailty

As of today, unlike BMD, there is no standardized tool or consensus definition available to measure or define frailty.

Fried et al. defined a frailty phenotype¹³⁹ by meeting three or more of the following criteria: low grip strength, low energy, slowed walking speed, low physical activity and unintentional weight loss.

Another approach to frailty is the use of a frailty-index where the number of deficits accumulated over time is counted. These variables can include disability, diseases, physical and cognitive impairments, psychosocial risk factors and geriatric syndromes (e.g. falls, delirium, and urinary incontinence).

While Fried's classification may be more appealing in a clinical setting due to its simplicity, the use of a frailty-index may be a more sensitive predictor of adverse health outcomes due to its finer graded risk.

Rationale for this thesis

In summary, vitamin D is an important regulator of calcium and phosphate homeostasis. Its integral role in providing sufficient calcium levels needed for healthy bones and muscles has been known for a long time. The discovery of Vitamin D receptors in most human cells has led to extensive research investigating the importance of vitamin D outside the musculoskeletal system.

Vitamin D insufficiency has been associated with increased fracture risk in the elderly. Insufficiency or deficiency in vitamin D is most likely a chronic condition in older adults. However, most studies so far relied on single time point measurements and did therefore not provide established information on the association between sustained insufficiency and the risk of fracture. The OPRA-cohort, in which 25OHD was measured at several time-points, gave us the opportunity to investigate the importance of “chronic” hypovitaminosis D and its association to fractures.

There is evidence from observational studies that low vitamin D levels increase the risk of mortality but comparatively little is known about what to regard as sufficient and optimal concentrations in regards to mortality. The long follow-up period of more than 15 years and 25OHD measurements at several time points allowed us to investigate this association as women advance from old to very old age. Moreover, since detailed information on fractures and comorbidities was available in all women, we were able to assess whether low vitamin D levels were associated with increased risk of mortality, independently of comorbidities, fractures and fracture-related frailty.

PTH and vitamin D are closely linked to each other. Data from some observational studies suggests that elevated PTH might contribute to mortality independently of vitamin D, at least in old and severely frail patients. PTH is assumed to increase with age, which in part might be related to ageing itself but the most important factors seem to be a declining kidney function and decreased vitamin D concentrations causing secondary hyperparathyroidism. However, whether reference ranges established in healthy, younger adults are applicable in old individuals and whether elevations of PTH above normal are independently associated with mortality or rather reflect a decline of kidney function and insufficient vitamin D status is not clear. The OPRA-cohort made it possible to

describe changes of PTH over time and to investigate its association to vitamin D, kidney function and mortality in relatively healthy, community-dwelling, women.

Vitamin D and its role in the pathogenesis and development of frailty is an area of increasing interest in the research community. However, our knowledge of the exact underlying mechanisms and its connection to vitamin D is clearly limited. Although, low vitamin levels have been associated with frailty previously, studies are limited due to relatively short follow-up periods or inclusion of individuals with slightly younger age. Another difficulty lies in the fact that we are still lacking a standardized measurement tool to assess frailty. In the OPRA-study, we were able to investigate the association between vitamin D and frailty at different time points up until very advanced age. Moreover, we assessed whether low vitamin D levels were associated with incident frailty at subsequent time points as well as accelerated progression of frailty. To do this, we created an OPRA-specific frailty index that was validated for its prediction of mortality.

Aims

The general aim of this thesis was to investigate whether a low vitamin D status was associated with increased adverse outcomes, including long-term risk of fracture, frailty and mortality in community-dwelling, relatively healthy, older women.

More specifically, we wanted to assess the risk of fracture associated with sustained hypovitaminosis D. Furthermore, we aimed to investigate the association between vitamin D and all-cause mortality taking into account comorbidities and fractures as important confounding factors. We also wanted to determine whether and for how long 25OHD can predict frailty. Additionally, we aimed to explore changes in parathyroid hormone with advancing age and to investigate whether elevations in parathyroid hormone levels were associated with frailty and mortality.

General hypotheses

1. Low vitamin D levels in older women are associated with
 - a. increased fracture risk
 - b. higher morbidity and mortality
 - c. higher frailty
2. The association between vitamin D and morbidity/mortality becomes stronger with increasing age.
3. PTH levels increase with age and elevated PTH levels are independently associated with higher mortality.

Specific research questions

STUDY I.

- Are low vitamin D levels associated with more hip fractures and major osteoporotic fractures during a 10 year follow up period?
- Does a single vitamin D measurement reflect the true vitamin D status over time as the women get older? (i.e how stable are vitamin D levels over time?)
- Is the number of women who sustain a fracture during 10 years increased if vitamin D levels are continuously low over a period of 5 years?

STUDY II.

- Is vitamin D an independent predictor of mortality? (i.e independent of fractures and coexisting morbidities?)
- Is sustained hypovitaminosis D associated with higher all-cause mortality?

STUDY III.

- Is parathyroid hormone increasing with age and if so, is the change independent of vitamin D and kidney function?
- Is PTH_r elevated above normal, associated with mortality?

STUDY IV.

- Are low vitamin D levels associated with prevalent frailty?
- Are low vitamin D levels associated with incident frailty?
- Is the progression of frailty over time associated with vitamin D?
- Does parathyroid hormone mediate the association between vitamin D and frailty?

Material and Methods

The OPRA cohort

The Osteoporotic Prospective Risk Assessment Cohort (OPRA) is a population based cohort of community-dwelling older women living in Malmö, South Sweden. Between 1995 and 1999, 1604 women, all aged 75, were randomly selected from population files. No exclusion criteria were applied. The women were prospectively followed for fractures and mortality until October 2012.

1044 women accepted the invitation and attended the baseline investigation. Reassessments were conducted after five and 10 years. These follow-up visits were attended by 715 and 382 women. 105 women died before age 80 and 205 women died between ages 80 and 85. 446 women were still alive at age 90 which was the end of the observational period (Figure 9.) The follow-up visits took place at around the same date and time as the initial visit.

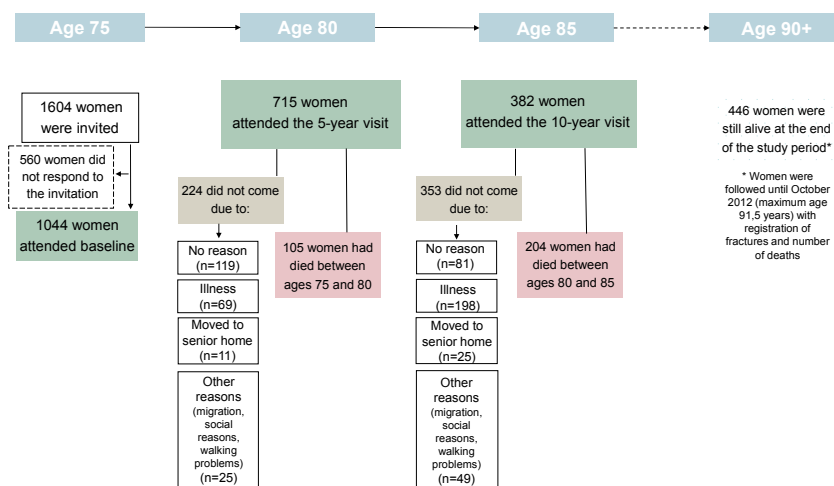


Figure 9. Participation and drop-outs in the OPRA-study

The assessments at each visit included measurements of bone mineral density measured by dual-energy X-ray absorptiometry (DXA), anthropometrics, blood and urine biochemistry and questionnaires on health, nutrition, medication and lifestyle.

Blood biochemistry

Blood samples were obtained non-fasting before noon and stored at -80°C before analyses. All analyses were performed at the Department of Clinical Chemistry, Skåne University Hospital, Sweden.

Vitamin D (25OHD)

25OHD was assessed using liquid chromatography mass spectrophotometry (LC-MS) linked to a High-Performance Liquid Chromatography (HPLC) system. The total coefficient of variation was four to six percent. The Department of Clinical Chemistry participated in the Vitamin D External Quality Assessment Scheme (DEQAS¹⁶¹) to ensure analytical reliability of performed measurements. For this study, serum samples were available for 1011 women at age 75, 642 at age 80 and 348 at age 85.

Parathyroid hormone (PTH)

PTH was assessed by Elecsys/Cobas PTH immunoassay (Roche Diagnostics) at ages 75 and 85 (CV 5-8%). At age 80, measurements were performed using Immulite 2000 Immunoassay Systems (Siemens Diagnostics, CV 5-7%). In order to assure inter-assay comparability, duplicate measurements were performed and corrections were made. Samples were available for 999 (age 75), 692 (age 80) and 348 (age 85) women.

Kidney function

Plasma creatinine (pCr) (IDMS traceable) and cystatin C (pCyC) were analysed at all time-points¹⁶². Estimated glomerular filtration rate (eGFR in $\text{mL}/\text{min}/1.73\text{m}^2$) was calculated using the Chronic Kidney Disease Epidemiology Collaboration equation (CKD-EPI) for females based on cystatin C and creatinine¹⁶². Samples were available for 963 (age 75), 683 (age 80) and 355 (age 85) women.

Other variables

Additionally, various variables and biomarkers including calcium, albumin and phosphate were assessed.

Comorbidities

The women's health status was assessed by information on comorbidities at the age of 80. Information on the following diagnoses was available: high blood pressure (defined as treatment with any hypertensive), cardiac infarction, angina, heart

failure, stroke, diabetes, asthma and allergies, emphysema, kidney disease, epilepsy, rheumatoid arthritis and osteoporosis. For the purpose of this analysis, these diagnoses were combined into four main categories (cardiovascular disease, respiratory disease, kidney disease and diabetes) along with osteoporosis. Additionally, we calculated the number of comorbidities (no comorbidity, one comorbidity and two or more comorbidities) in order to investigate the influence of higher disease burden.

Fracture assessment

Incident fractures

Information on fractures from age 75 until age 90 was continuously registered and obtained through X-ray files at the Radiology Department, Skåne University Hospital, Malmö by using the personal identification number allotted to every Swedish citizen. This department, where all X-ray files have been saved since the beginning of the last century, serves the Department of Orthopedics which is the only unit treating adult and pediatric fractures in the catchment area. Loss to follow-up was therefore exceptionally low¹⁶³.

For this project, information on hip fractures and major osteoporotic fractures was obtained. The assessment of major osteoporotic fractures included fracture sites that are used in the Fracture Risk Assessment Tool (FRAX®; <http://www.shef.ac.uk/FRAX>), i.e. hip fractures, vertebral fractures, shoulder fractures and fractures of the distal radius.

Pathological fractures and high-energy fractures were excluded from the analyses.

During the study period, 349 women suffered 452 fractures at major osteoporotic sites (hip n=130, vertebra n=152, distal radius n=100, shoulder n=70). The majority of fractures occurred after the age of 80 (hip 61%; major osteoporotic fractures 60%).

The association between vitamin D and fractures was tested over a period of 5 and 10 years using both single vitamin D measurements and, in order to investigate the association between “chronic” hypovitaminosis D and fractures, duplicate measurements (Figure 10.)

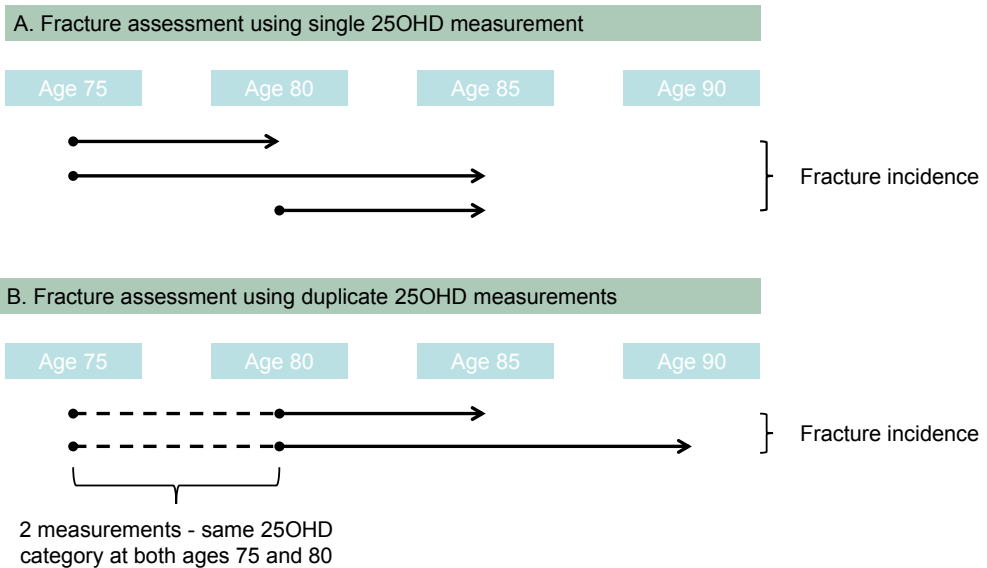


Figure 10. Fracture assessment using single and duplicate 25OHD measurements

Single time point measurements of 25OHD at age 75 and 80 were used to investigate their association with fractures between ages 75-80, 75-85 and 80-85. Duplicate measurements at both ages 75 and 80 were used to investigate the association between sustained hypovitaminosis D and fractures between ages 80-85 and 80-90. Women were categorized according to their 25OHD levels as high (>75 nmol/L), intermediate (50-75 nmol/L) and low (<50 nmol/L).

Prevalent fractures at age 75

Information on fractures that had occurred prior to inclusion was obtained through questionnaires¹⁶⁴ but was not used in this project.

Mortality assessment

Information on date of death was collected through the Swedish National Population Register. For the purpose of this project, mortality was assessed prospectively from age 75 until the end of follow-up at around age 90 (October 2012). Mortality analyses for 25OHD were once again based on single or duplicate measurements as shown in Figure 11. Women were categorized according to their 25OHD levels as high (>75 nmol/L), intermediate (50-75 nmol/L) and low (<50 nmol/L).

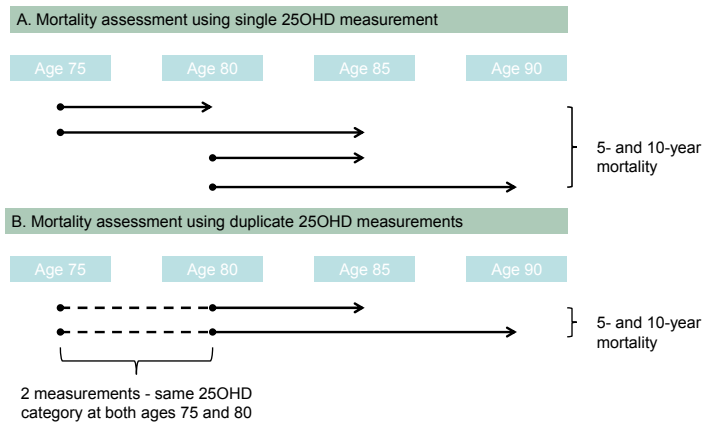


Figure 11. Mortality assessment using single and duplicate 25OHD measurements

Single time point measurements of 25OHD at age 75 and 80 were used to investigate their association with mortality between ages 75-80, 75-85, 80-85 and 80-90. Duplicate measurements at both ages 75 and 80 were used to investigate the association between sustained hypovitaminosis D and mortality between ages 80-85 and 80-90.

The association between PTH and mortality was assessed using the currently defined reference intervals for PTH as shown in Figure 12. Women were categorized according to their PTH levels as normal (1.6-6.9 pmol/L) and elevated (>6.9 pmol/L). Additional analyses were performed using quartiles of PTH.

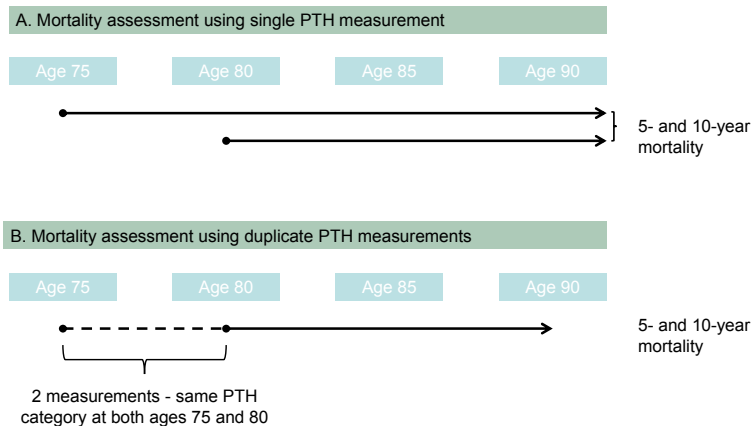


Figure 12. Mortality assessment using single and duplicate PTH measurements

Single time point measurements of PTH at age 75 and 80 were used to investigate their association with mortality between ages 75-90 and 80-90. Duplicate measurements at both ages 75 and 80 were used to investigate the association between continuously elevated PTH and mortality between ages 80-90.

Frailty assessment

In this project, a frailty index specific for the OPRA-cohort, consisting of 10 variables that were available at all time-points, was used (Table 6.). This index was tested previously against indices including 40 and 15 variables and the correlation between them was high. The frailty index (FI) was created using the general principles described by Searle et al.¹⁶⁵. It covers a number of physiological domains e.g. mobility, strength, co-ordination and poly-medication. The index represents, for each OPRA participant, the number of ‘deficits in health’. For binary variables, the presence or absence of a deficit was coded as 1 or 0 respectively. To dichotomize continuous variables – defining if a deficit was present or not - we used either clinically relevant cut-points or identified threshold values by plotting the variable against an interim frailty index, a method described by Searle et al. Categorical variables containing more than two categories were scored 0-1 according to the numbers of categories (e.g. high/medium/low = 1.0/0.5/0.0). The frailty index was calculated by dividing the number of deficits present by the total number of deficits examined; giving a score from 0.0-1.0, where a higher score indicates a higher frailty status. Where an individual had missing information for a particular variable, the total deficits were reduced by one.

Our modified frailty index uses 10 variables. To validate its predictive ability of mortality, Cox regression analysis was performed using death status at 5 and 10-year follow-ups as the outcome. Comparing individuals in the highest quartile (Q4) of frailty to those in the lowest (Q1), the 5-year HR_{unadjusted} was 3.65 (2.04-6.53), $p < 0.001$ and 10-year HR_{unadjusted} 3.51 (2.50-4.92), $p < 0.001$.

Table 6. The OPRA-frailty-index (10 variables)

Daily activity level	Walking independent=0; Walking with walking aid=0.5; Bedbound=1
Body mass index (kg/m ²)	23-33=0; <23 and >33=1
Walking speed 2x15m (m/s)	≥1.2=0; <1.2=1
Walking 2x15m (Number of steps)	<54=0; ≥54=1
Muscle strength (Knee extension 90°) (Nms)*	≥213=0; <213=1
Diabetes	No=0; Yes=1
Severe disease	No=0; Yes=1
Using five or more medications	No=0; Yes=1
Self-reported fall risk	Categories 1 (lowest)-5 (highest): 0, 0.25, 0.5, 0.75, 1.0
Diseases affecting balance	No=0; Yes=1

*Voluntary maximal, isometric muscle strength of the right knee measured by Biodex computerized dynamometer and recorded in Newton meter seconds (Nms)

Statistical analyses

IBM SPSS Statistics® version 20-22 was used for all statistical analyses.

Descriptive data are presented as absolute numbers, percentages and means \pm standard deviations (SD). P-values ≤ 0.05 were regarded as nominally significant.

Vitamin D and PTH data were tested for normality using the Shapiro-Wilks test. The women were categorized according to their vitamin D status as either low (<50 nmol/L), intermediate (50-75 nmol/L) and high (>75 nmol/L) or insufficient (<50 nmol/L) and sufficient (>50 nmol/L). Additionally, analyses were performed using quintiles as well as z-scores of 25OHD.

PTH levels between 1.6 and 6.9 pmol/L were classified as normal and >6.9 pmol/L as elevated above normal range. Additional categorizations were made using quartiles of PTH as well as percentage increases over time ($<1\%$, 1-10%, 11-20%, 21-30%, 31-40%, 41-50% and 51% and above).

Pearson correlation coefficient (r) was used to test correlations between 25OHD, PTH and relevant biomarkers.

Study I

Regression analysis was used to identify independent predictors of fracture and included 25OHD, BMD, body mass index (BMI), bisphosphonate use, smoking and physical activity.

The Pearson Chi-2 test was used to compare categories of 25OHD and fracture incidence. Hazard ratios (HR) and 95% confidence intervals (CI) for fractures were calculated from Cox proportional hazard models. HR's were calculated unadjusted and adjusted for smoking, bisphosphonate use and physical activity. Additionally, Poisson distribution was used to estimate fracture rates per 1000 person years.

Study II

Independent predictors of mortality were identified by multiple regression analysis. The following variables were included: body mass index, physical activity, smoking, osteoporosis (femoral neck T-score <-2.5), and comorbidities (CVD, respiratory disease, kidney disease, diabetes mellitus).

Mortality risk was calculated using Cox proportional hazard models. HR and CI were estimated in the different 25OHD categories using the highest category as reference. HR were calculated in the whole study population as well as in a subgroup of fracture-free women. Diagnostics of collinearity between fracture, vitamin D, and mortality were performed, and the variance inflation factor values indicated that,

although vitamin D and fracture were highly correlated, a correlation between fracture and mortality was unlikely to be problematic.

Study III

The relationship between PTH and mortality was investigated by Cox proportional hazard models to estimate HR and CI using normal and elevated PTH categories and quartiles of PTH.

HR's were presented unadjusted and adjusted for 25OHD, kidney function, phosphate, smoking, comorbidities (only age 80) and calcium and vitamin D supplementation.

Study IV

General linear models were used to compare frailty indices in different 25OHD and PTH categories.

Multinomial regression analysis was used to test the association between 25OHD and individual variables included in the frailty index and to calculate odds ratios (OR). All analyses were adjusted for relevant covariates. For 25OHD, these variables included eGFR and vitamin D supplementation. PTH was adjusted for 25OHD, eGFR and comorbidities (at age 80).

Power analysis

The OPRA cohort study was originally designed as bone density and fracture study.

As such, power analyses were performed to determine the sample size needed to detect a difference in bone mineral density by $0,056 \text{ g/cm}^2$ and similarly one standard deviation change in the bone turnover marker carboxylated osteocalcin (mean value of $13.1 + 19.1 \text{ ng/mL}^{166}$).

The size of the population was chosen because of the variance of biochemical markers and their estimated ability to discriminate between individuals with and without hip fracture. It was estimated, that 60 women of the cohort of 75-year-olds would sustain a hip fracture during a 5-year study period. A number of 50 was chosen to take account for drop-outs. To obtain an 80% power at a significance level of 5% to detect a 30% difference in carboxylated osteocalcin, serum was needed from 50 hip fracture patients to be compared with 600 women without fracture. Thus, the initial sample size of 1,000 women was considered to be sufficient.

Results

General results

Characteristics of the OPRA cohort

Table 7 shows the most relevant characteristics of the OPRA cohort based on vitamin D categories at ages 75, 80 and 85.

Supplementation and comorbidities

At age 75, 65 women (6%) were taking vitamin D supplements. The number increased to 113 (16%) at age 80 and 121 (32%) at age 85.

At age 75, the proportion of women reporting regularly walking outdoors, with or without walking aids was 96% (n=272) in the low and 99% (n=233) in the high 25OHD category. At ages 80 and 85, these proportions were similar to the ones found at baseline (Age 80: low 92% [n=92] and high 99% [n=299]; Age 85: low 95% [n=40] and high 99% [n=177]).

The prevalence of morbidities was similar regardless of whether women had low or high 25(OH)D levels, with the exception of osteoporosis which was more prevalent (~3x higher) in women with high levels.

Table 7. Characteristics of the OPRA cohort

This table shows characteristics of the OPRA cohort at ages 75, 80 and 85 based on 25OHD categories

	25OHD Category					
	LOW		INTERMEDIATE		HIGH	
AGE 75	n=284 (28%)		n=491 (49%)		n=236 (23%)	
S-25OHD (nmol/L)	40	(8)	62	(7)	88	(12)
Vitamin D use (<i>Number(%)</i>)	7	(3)	32	(6)	22	(9)
Calcium use (<i>Number(%)</i>)	14	(5)	33	(7)	20	(8)
Bisphosphonate use (<i>Number(%)</i>)	6	(2)	12	(2)	13	(5)
BMI (kg/m ²)	27	(5)	26	(4)	25	(4)
S-PTH (pmol/L)	5.2	(2.2)	4.6	(2.0)	4.1	(1.8)
S-Calcium (mmol/L)	2.4	(0.1)	2.4	(0.1)	2.4	(0.1)
S-Phosphate (mmol/L)	1.1	(0.2)	1.1	(0.2)	1.1	(0.2)
eGFR (mL/min/1.73m ²)	70	(15)	69	(16)	67	(14)
AGE 80	n=101 (16%)		n=233 (36%)		n=306 (48%)	
S-25OHD (nmol/L)	38	(10)	63	(7)	102	(22)
Vitamin D use (<i>Number(%)</i>)	4	(4)	18	(8)	74	(24)
Calcium use (<i>Number(%)</i>)	5	(5)	34	(15)	114	(37)
Bisphosphonate use (<i>Number(%)</i>)	2	(2)	7	(3)	27	(9)
BMI (kg/m ²)	26	(4)	26	(4)	26	(4)
S-PTH (pmol/L)	5.4	(3.9)	4.6	(3.0)	4.0	(3.1)
S-Calcium (mmol/L)	2.4	(0.1)	2.4	(0.1)	2.4	(0.1)
S-Phosphate (mmol/L)	1.1	(0.1)	1.1	(0.1)	1.1	(0.1)
eGFR (mL/min/1.73m ²)	61	(14)	62	(14)	60	(15)
AGE 85	n=42 (12%)		n=127 (37%)		n=179 (51%)	
S-25OHD (nmol/L)	38	(9)	64	(7)	99	(17)
Vitamin D use (<i>Number(%)</i>)	3	(7)	18	(14)	69	(38)
Calcium use (<i>Number(%)</i>)	3	(7)	34	(27)	111	(62)
Bisphosphonate use (<i>Number(%)</i>)	1	(2)	11	(9)	32	(18)
BMI (kg/m ²)	25	(4)	26	(4)	25	(4)
S-PTH (pmol/L)	6.8	(6.8)	5.5	(4.0)	4.5	(4.0)
S-Calcium (mmol/L)	2.3	(0.1)	2.3	(0.1)	2.4	(0.1)
S-Phosphate (mmol/L)	1.1	(0.1)	1.1	(0.1)	1.1	(0.1)
eGFR (mL/min/1.73m ²)	54	(14)	53	(15)	53	(13)

Values are mean (SD); 25OHD categories are Low (<50 nmol/L); Intermediate (50-75 nmol/L); High (>75 nmol/L)

Vitamin D status over time

The mean 25OHD level at baseline was 62 nmol/L (SD 19) which can be considered relatively high given the age of participants and latitude of the study location. Although a statistically significant seasonal variation was observed, it was not consistent over time and its clinical relevance is debatable (The seasonal variation of vitamin D is described in more detail in the appendix II, pages 79-81). Women

classified as being in the ‘high’ category at age 75 generally continued to have high levels at subsequent measuring points, even after excluding those taking vitamin D supplements. Of the women who were classified as having the lowest levels at age 75, a similar proportion (42%) continued to have low levels or moved to the ‘intermediate’ category. Individuals lost to follow-up in the first 5 years were more likely to have been in the low 25OHD category (42%; n=120) than the intermediate (37%; n=182) or high categories (34%; n=81). The distribution of women in the respective 25OHD categories is presented graphically in Figure 13.

The mean 25OHD concentration during follow-up increased to 78 nmol/L at age 80 and 79 nmol/L at age 85. The exact reason for this is unknown to us but we have eliminated methodological errors. The increasing number of women taking vitamin D supplements and the fact that women with higher 25OHD levels are likely be healthier and thus able to participate at subsequent time points may be contributing factors.

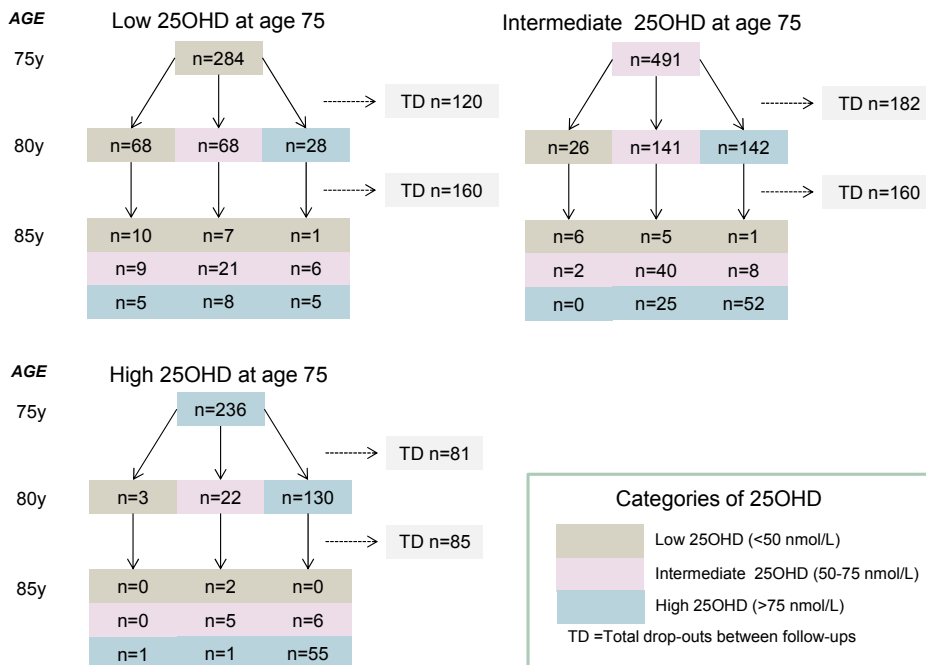


Figure 13. Distribution of women within categories of 25OHD

This figure shows the number of women within the 25OHD categories (low, intermediate and high) at baseline and during follow up as well as drop-out rates in the respective categories.

Study I – Vitamin D Insufficiency Over 5 Years is Associated with Increased Fracture Risk – An Observational Cohort Study of Elderly Women

Context

While the association between a single vitamin D measurement and hip fracture has been investigated in a number of studies, the consequence of prolonged vitamin D insufficiency in this age group has not been adequately investigated. We therefore used sequential assessments of serum 25OHD at ages 75 and 80 and investigated if continuously low 25OHD levels were associated with increased long-term fracture risk.

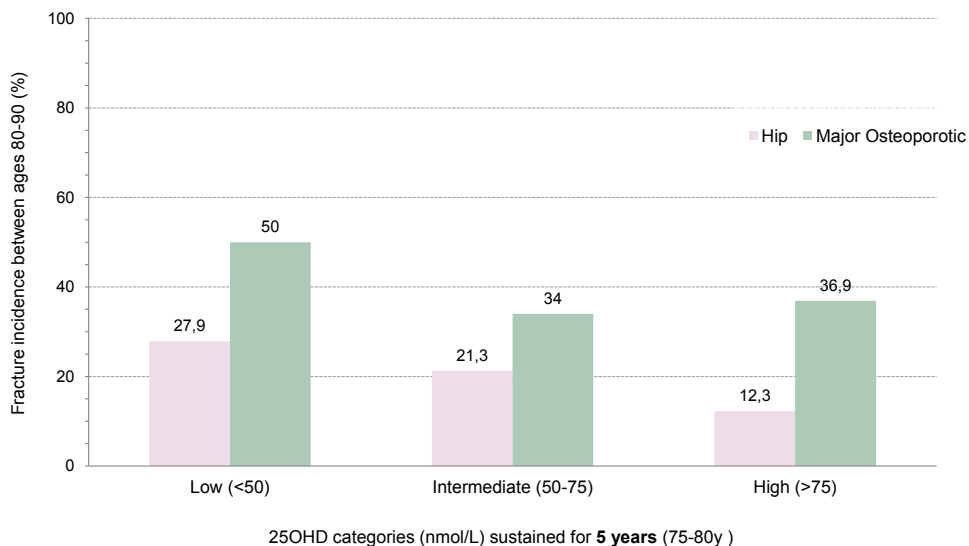
Subjects and Methods

For this study, data from baseline (age 75, n=1044) and 5-year follow-up visits (age 80, n=715) were used. Vitamin D (25OHD) was available in 987 and 640 women. Women were categorized according to their vitamin D status as: low (<50 nmol/L), intermediate (50-75 nmol/L) and high (>75 nmol/L). Incident fracture data was collected with a maximum follow-up to 90 years of age. Cox proportional hazards models were used to calculate 5- and 10-year fracture-risk.

Results

During the full follow-up period, 349 women suffered 452 fractures at major osteoporotic sites (hip n=130, vertebra n=152, distal radius n=100, shoulder n=70). The majority of fractures occurred after the age of 80 (hip 61%; major osteoporotic fractures 60%).

Fractures within 5 years: The hip fracture incidence between ages 80 and 85 was higher in women who had continuously low 25OHD at both baseline and 5-years (22.2% (Low) vs 10.5% (Intermediate) vs 6.6% (High); $p=0.028$ and 0.003).



	Low vs High	Low vs Intermediate	Intermediate vs High
Hip	$p=0.006$	$p=0.186$	$p=0.035$
Major Osteoporotic	$p=0.053$	$p=0.020$	$p=0.356$

Figure 14. Fracture incidence between ages 80-90 based on duplicate 25OHD measurements

This figure shows the incidence of hip and major osteoporotic fractures in women who maintained in the same 25OHD category from age 75 to age 80.

50% of women in the continuously low group compared to 28% and 34% in the higher 25OHD groups suffered a major osteoporotic fracture ($p=0.004$ and 0.029).

Fractures within 10 years: Women who had continuously low 25OHD levels also had a higher incidence of fractures in the long term (80-90y), with a hip fracture incidence that was more than double that of women in the high category (27.9% v 12.3%; $p=0.006$). Even women who would be considered vitamin D sufficient with intermediate levels (50-75 nmol/L), had a higher fracture incidence (21.3% v 12.3%; $p=0.035$) (Figure 14.). The 10-year relative risk of hip fracture was almost 3 times higher for women in the low 25OHD category compared to the high category (HR 2.7 [1.4–5.3], $p=0.003$). The incidence of major osteoporotic fractures was also higher among women who had continuously low compared to intermediate 25OHD levels (50 vs. 34 %; $p=0.020$), but did not reach statistical significance compared with the high category (50 vs. 36.9 %; $p=0.053$). The risk of sustaining a major osteoporotic fracture was almost double that of women in the intermediate and high categories (low vs. high: HR 1.7 [1.1–2.6], $p=0.023$; low vs. intermediate: HR 1.8 [1.2–2.8], $p=0.008$)

Conclusions

In this prospective population-based study of Swedish women aged 75, we found that prolonged hypovitaminosis D was associated with a higher risk of hip fractures and major osteoporotic fractures for up to 10 years. Women who maintained 25OHD levels above 50 nmol/L, had a lower incidence of major osteoporotic fractures but in the eldest women, 25OHD levels above 75nmol/L might be needed in order to achieve a significant risk reduction of hip fractures.

Study II – Hypovitaminosis D In Elderly Women Is Associated With Long And Short Term Mortality – Results From The OPRA Cohort

Context

Inadequate vitamin D levels have been found to be associated with mortality. However, comparatively little is known about the optimal vitamin D concentration in regards to improved survival, independent of comorbidities and fracture-related frailty. The primary objective of this study was therefore to investigate the relationship between hypovitaminosis D and all-cause mortality in community-dwelling older women. In a secondary analysis, this association was tested in a fracture-free and presumably less frail subgroup of women.

Subjects and Methods

1044 women were included at baseline, 715 women (age 80) and 382 women (age 85) attended the 5- and 10-year follow-up visits. From the cohort, a subgroup of women who did not sustain any type of fracture during the entire observation period (n=439) was selected in order to test whether similar associations with mortality were observed in the absence of fracture-related frailty. Vitamin D levels at each visit were categorized as low (<50 nmol/L), intermediate (50-75 nmol/L) and high (>75 nmol/L). Mortality risk in the primary and secondary analyses were calculated using Cox proportional hazard models.

Results

Based on a 25OHD measurement at age 75, mortality risk within 10 years of follow-up was significantly greater for women in the low 25OHD category than for women with high 25OHD (HR 1.4 [1.0-1.9], $p=0.04$). This increased risk remained even after adjustment for smoking (HR 1.4 [1.0-2.0], $p=0.03$) and a diagnosis of osteoporosis (HR 1.4 [1.0-1.9], $p=0.04$).

Between ages 80 and 90, once again, all-cause mortality was significantly higher in women with low 25OHD levels compared to women with high levels unadjusted and after adjustment for comorbidities (HR 1.8 [1.3-2.4], $p < 0.001$ and HR_{adj} 1.9 [1.4-2.6], $p < 0.001$). Of all the comorbidities, osteoporosis had the highest impact on mortality, but even after excluding women with osteoporotic fracture during the entire observational period, the risk of dying associated with low 25OHD remained increased (HR_{unadj} 1.8 [1.2-2.7], $p = 0.002$ and HR_{adj} 1.7 [1.2-2.5], $p = 0.006$) (Figure 15.).

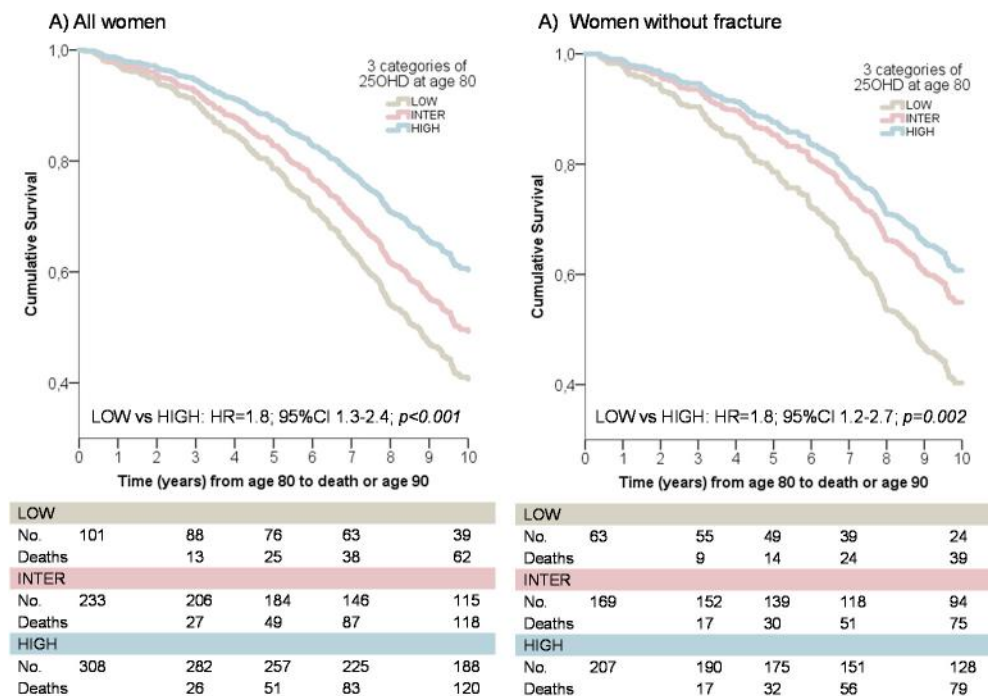


Figure 15. 10-year mortality risk in A) all women and B) women without fracture
 This figure shows the risk of dying between ages 80 and 90 years based on a single 25OHD measurement at age 80 years. Additionally, the number of deaths that occurred until ages 83, 85, 87 and 90 years is shown, grouped by categories of 25OHD (low, intermediate and high).

Conclusions

In this prospective cohort study of Swedish women, all aged 75 at inclusion and followed into their nineties, hypovitaminosis D was associated with increased all-cause mortality for up to ten years, irrespective of comorbidities. Furthermore, mortality risk was also higher in women without osteoporotic fractures, suggesting that the relationship with vitamin D status is at least in part independent of health status and fracture-related frailty.

Study III – PTH in Community Dwelling Older Women - Elevations Are Not Associated with Mortality

Context

Our understanding of age-related changes of parathyroid hormone (PTH) in the aging population is limited. Whether the normal reference range is applicable even in the oldest old is unclear and so is the clinical implication of elevated PTH in this age group. In this study of community-dwelling women aged 75 and followed for up to 15 years, we described changes of PTH over time and its associations to related biomarkers such as 25OHD and kidney function (eGFR). Furthermore, we investigated the association between elevated PTH and mortality.

Subjects and Methods

Participants were women from the Swedish OPRA cohort. Serum PTH was available for 999 of 1044 who attended baseline (age 75), 692 of 715 who attended follow-up at age 80 and 348 out of 382 who attended follow-up at age 85. PTH levels between 1.6 and 6.9 pmol/L were considered being within the normal reference range (NRR). 25OHD was available for 1011 (75y), 642 (80y) and 348 women (85y). Estimated glomerular filtration rate (eGFR in mL/min/1.73m²) used the CKD-EPI equation for females based on cystatin C and creatinine and was available for 963 (75y), 683 (80y) and 355 women (85y). Correlations between PTH and related biomarkers were estimated by Pearson's correlation coefficient (r) and mortality risk was calculated using Cox proportional hazards models.

Results

At age 75, the majority of women (88%, n=877) had PTH within normal reference range (NRR) (1.6-6.9 pmol/L). PTH levels below 1.6 pmol/L were observed in 13 women; their eGFR, 25OHD and phosphate were similar to those with PTH in the NRR ($p=0.24-0.53$). The remainder (11%) had PTH elevated above NRR and these women had lower eGFR (60 vs 70 mL/min/1.73m², $p<0.001$) and 25OHD (54 vs 63 nmol/L, $p<0.001$) compared to women with normal PTH.

At age 80, the proportion of women with PTH elevated above NRR was almost doubled (19%). These women had lower eGFR (53 vs 63 mL/min/1.73m², $p<0.001$) and 25OHD (70 vs 80 nmol/L, $p=0.001$) compared to those in the NRR. Contrary to the observations at 75y, phosphate was also lower (1.06 vs 1.10 mmol/L, $p=0.004$)

Between ages 75 and 80, PTH increased in 60% (n=390) of subjects over the 5-year period. Even so, the majority of these (64%; n=250) remained within NRR despite PTH increases of up to 50% above baseline values. These women had significantly

lower 25OHD (74 ± 29 vs 83 ± 32 nmol/L, $p=0.001$) compared to those with stable PTH, although eGFR, phosphate and calcium remained unaltered ($p=0.07-0.88$). For those women with PTH increases of more than 50% compared to baseline (36%), mean PTH was now above NRR (7.1 ± 3.3 pmol/L). This elevation was associated with reduced kidney function (eGFR 56 ± 15 vs 61 ± 15 mL/min/1.73m², $p=0.002$) but no further reduction in 25OHD (77 ± 27 vs 74 ± 29 nmol/L, $p=0.31$) when compared to women whose PTH only increased by up to 50%. Phosphate and calcium remained unaltered ($p=0.23-0.99$).

By age 85, half of the women at age 85 years had stable or decreased PTH levels (51%; n=169). Amongst those women whose PTH had increased with age, we again observed that the majority (64%; n=105) remained within NRR despite increases of up to 50% above baseline values (Figure 16.). Associations to 25OHD, eGFR, calcium and phosphate were similar to the first 5 years of follow-up.

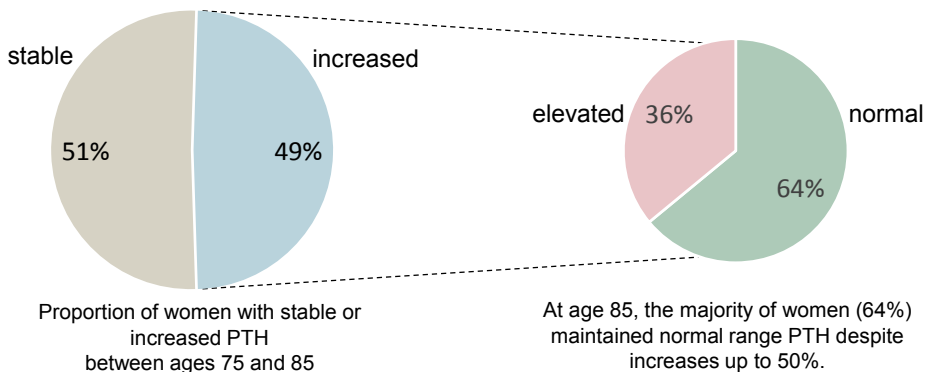


Figure 16. Proportion of women with stable or increased PTH between ages 75 and 85 years. This figure shows the proportion of women who had stable or increased PTH levels during follow-up (ages 75-90 years). Among those who increased their PTH, the proportion of women with PTH levels within and above the normal reference range is shown.

Measured at age 75, women with PTH elevated above NRR had a higher mortality risk (HR 1.4 [1.1-1.8], $p=0.007$). However, after adjustment for confounding factors (25OHD, kidney function, phosphate, smoking, comorbidities (only age 80) and calcium and vitamin D supplementation (n=91 at baseline), results became insignificant.

Conclusions

In this population based study of community-dwelling older women, the majority maintained PTH levels within normal range despite substantial increases with only one or two in ten above. Elevated PTH was not independently associated with mortality but merely reflected a low 25OHD status and impaired kidney function.

The decline in kidney function seems to be the predominant factor driving PTH upwards with increasing age.

Study IV – Association Between Vitamin D and Frailty in Community-Dwelling Older Women

Context

Low vitamin D levels may increase the risk of frailty, a recognized geriatric syndrome with a functional decline of multiple physiological systems that embodies an increased risk for negative health outcomes. However, most studies have either included younger individuals or had relatively short follow-up periods (~3 years). Thus it is unclear whether 25OHD can be used as long-term risk marker for frailty and whether the association is consistent with advancing age.

In this study, we primarily investigated the association between low 25OHD and prevalent and incident frailty as well as progression of frailty between ages 75 and 85. In a secondary analysis, we also assessed the association between PTH and frailty.

Subjects and Methods

Participants were women from the Swedish OPRA cohort. Using the OPRA-specific frailty-index, frailty was assessed at ages 75 (n=1044), 80 (n=715) and 85 (n=382). Women were categorized according to their 25OHD levels as insufficient (<50 nmol/L) and sufficient (\geq 50 nmol/L). The association between 25OHD, PTH and frailty was tested using General Linear Models at the time of measurement (prevalent frailty) as well as at prospective time-points, i.e. ages 80 and 85 (incident frailty). PTH levels between 1.6 and 6.9 pmol/L were defined as the normal reference range. Multinomial regression analyses were performed to calculate odds ratios for individual variables in the frailty index in order to further explore the relation between 25OHD and frailty.

Results

Cross-sectionally, at both ages 75 and 80, women with insufficient 25OHD were more frail compared to women with sufficient 25OHD (0.23 vs 0.18; $p<0.001$ and 0.32 vs 0.25; $p=0.001$). Longitudinally, insufficient 25OHD at age 80 was also associated with subsequent frailty 5 years later (0.41 vs 0.32; $p=0.011$) (Table 8.). Interestingly, accelerated progression of frailty was not associated with lower 25OHD, neither was 25OHD >75nmol/L additionally beneficial with regards to frailty.

Within the frailty-index, variables that were associated with 25OHD were those related to muscle function. At age 75, 25OHD insufficient women had higher odds of having lower muscle strength (OR 1.40 [1.01-1.95], $p=0.042$), being in need of walking aids (OR 1.67 [1.04-2.68], $p=0.032$), being bedbound (OR 4.13 [1.67-10.22], $p=0.002$), having a lower walking speed (OR 1.94 [1.43-2.63], $p<0.001$) and taking more steps to cover the same distance (OR 1.74 [1.24-2.43], $p=0.001$). At age 80, similar odds ratios were found regarding the need of walking aids, being bedbound, having a lower walking speed and taking more steps to cover the same distance. In contrast to age 75, this was not reflected in the odds of having a lower muscle strength (OR 1.2 [0.8-1.9], $p=0.294$).

No association between 25OHD and frailty was observed at age 85.

At age 75, women with PTH elevated above the normal range (>6.9 pmol/L) were more frail compared to women with normal PTH concentrations (0.25 vs 0.19; $p<0.001$). Adjustment for 25OHD and plasma calcium did not alter this association. However, after additional adjustment for kidney function (eGFR), significance was lost.

No association between PTH and frailty was observed at ages 80 and 85.

Table 8. Association between 25OHD and frailty

This table shows the association between 25OHD and frailty at the time of measurement at ages 75, 80 and 85 years (prevalent frailty) as well as at subsequent time-points during follow-up (i.e. ages 80 and 85 years; incident frailty). FI=frailty index; p -values comparing insufficient and sufficient categories.

	FI	p	FI	p	FI	p
	Age 75y		Age 80y		Age 85y	
25OHD measured at age 75y						
Insufficient (<50 nmol/l)	0.23 (n=284)	<0.001	0.28 (n=179)	0.122	0.34 (n=91)	0.979
Sufficient (≥50 nmol/l)	0.18 (n=727)		0.26 (n=520)		0.34 (n=286)	
25OHD measured at age 80y						
Insufficient (<50 nmol/l)			0.32 (n=101)	0.001	0.41 (n=37)	0.011
Sufficient (≥50 nmol/l)			0.25 (n=539)		0.32 (n=285)	
25OHD measured at age 85y						
Insufficient (<50 nmol/l)					0.38 (n=42)	0.187
Sufficient (≥50 nmol/l)					0.34 (n=306)	

Among the least frail women, 82% attended the follow-up visit at age 80 and 6% died during the first 5 years. In the most frail group, only 34% remained in the study 5 years later and 34% had died prior to the first follow up visit.

Conclusions

In this prospective observational cohort study of women, aged 75, vitamin D insufficiency was associated with higher frailty scores in all but the oldest old. Low 25OHD was associated with impaired muscle function indicated by impaired muscle strength, the need of walking aids and lower walking speed. This study lends further evidence towards the importance of sufficient vitamin D levels for maintaining musculoskeletal health in advanced age. PTH was not independently associated with frailty.

Discussion

Main findings

In this project of community-dwelling women, aged 75 and followed for more than 15 years, low vitamin D levels were associated with increased fracture risk, higher grade of frailty and increased mortality. Elevated parathyroid hormone was not an independent predictor of frailty or mortality.

Vitamin D and the risk of fractures

The aim of the first study was to expand our knowledge of the association between low vitamin D levels in older women and the risk of fractures. More specifically, we examined women with sustained, or prolonged, vitamin D insufficiency over 5 years and investigated its association to hip and major osteoporotic fractures.

Our main finding was that chronic vitamin D insufficiency was associated with a higher risk of hip fractures and major osteoporotic fractures.

These results are in part confirmative of previous findings^{87,167}. However, since most previous studies measured 25OHD at a single time point, it has not been confirmed that this provides a correct assessment of the vitamin D status. Assuming that vitamin D insufficiency in the elderly most likely is a chronic condition, using duplicate measurements allowed us to investigate the importance of "chronic" hypovitaminosis D in older women based on established information rather than assumptions.

There is an apparent discrepancy between observational data indicating an association between low vitamin D levels and increased fracture risk^{86,88,89} and interventional trials where results are less consistent^{91,94}. These trials often could not convincingly demonstrate a fracture preventive effect of vitamin D supplementation. Possible explanations for this could be inconsistent dosage of vitamin D, poor compliance, lack of laboratory tests to confirm vitamin D deficiency and thus inclusion of vitamin D sufficient individuals as well as lack of clear cut-off values on how to define vitamin D sufficiency and insufficiency. On the other

hand, uncontrolled confounding in observational studies could have influenced the outcomes as well. The OPRA-cohort consists of relatively healthy, independently living women with few comorbidities and thus less confounding.

The long follow-up time of more than 15 years with laboratory samples available at several time-points gave us the opportunity to follow these women from old to very old age and to determine whether the association between low vitamin D and fracture risk is consistent over time. Interestingly, while levels above 50 nmol/L were associated with a lower risk of hip fractures and major osteoporotic fractures between ages 80 and 85, higher levels (>75 nmol/L) were needed in women aged 85 and above in order to achieve a significant risk reduction of hip fractures. This could indicate that the need of vitamin D in order to maintain bone health increases with increasing age.

Vitamin D and the risk of dying

Here, we investigated the association between vitamin D and all-cause mortality between ages 75 and 90 years.

Our main finding was that women with low vitamin D levels (<50 nmol/L) had a substantially higher risk of dying within 10 years from the time of measurement compared to women considered as having optimal vitamin D concentrations (>75 nmol/L); importantly, this was independent of coexisting morbidities .

In the first subgroup analysis, we once again investigated women with “chronic” hypovitaminosis D. The results mirrored those obtained in the primary analysis.

Since low vitamin D levels were associated with increased fracture risk in these women, we performed a further subgroup-analysis in fracture-free women, in order to rule out fracture related frailty and mortality as confounding factor. The mortality risk in these vitamin D insufficient women was similar to those in the whole study population indicating that the association between low vitamin D and mortality is unlikely to be solely mediated by comorbidities and fractures.

Once again, there is a discrepancy between data from observational studies implying associations between low vitamin D and increased mortality¹⁶⁸ and results from randomized controlled trials failing to show benefits of supplementation¹⁶⁹. It is worth mentioning that most randomized trials conducted so far were originally designed as fracture outcome studies and did not have mortality as primary outcome measure.

The causal pathway between vitamin D and mortality cannot be proven due to the observational nature of this study. Reverse causation, meaning that low vitamin D

levels are caused by diseases, should be considered possible. However, the association between vitamin D and mortality was consistent over a period of 10 years and remained even after adjustment for comorbidities and fractures. This indicates that the contribution of hypovitaminosis D to mortality is, at least in part, independent from other relevant diseases and medical conditions commonly seen in the aging population¹⁷⁰.

The association between vitamin D and frailty

In this study, we investigated the association between vitamin D and frailty between ages 75 and 90 using a quantitative frailty-index.

The main finding of this study was that vitamin D insufficiency was associated with higher frailty at ages 75 and 80 which is in line with results from previous studies with slightly younger participants or with shorter follow-up periods^{158,171,172}.

In contrast to these studies, a low vitamin D status was not associated with accelerated progression of frailty nor was an increase of 25OHD >75 nmol/L further beneficial in regards to preventing frailty. Moreover, only at age 80 was vitamin D insufficiency associated with increased frailty at subsequent measurement. The relatively long time between follow-ups (5 years) may have affected the frailty predictive value of vitamin D.

The association between vitamin D and frailty was not consistent over time. The relatively minor contribution of vitamin D to frailty overall and an increasing number of comorbidities with advanced age could possibly explain why the association between vitamin D and frailty was non-existent in the oldest women.

Our knowledge of the exact pathway in the development of frailty is clearly limited. Chronic inflammation and activation of the immune system seem to play an integral role in its pathogenesis¹⁷³. These findings could provide a possible link to vitamin D through a potential modulatory effect on inflammatory markers and immune cells¹⁷⁴.

Muscle weakness represents the most common first manifestation of frailty¹⁴⁶. We found that low vitamin D levels were indeed associated with impaired muscle strength and muscle function, indicating that, in a clinical setting, measurement of 25OHD could potentially be a useful risk marker in the assessment and early identification of frailty.

The fact that progression of frailty over time was not correlated to the women's vitamin D levels raises the question whether supplementation in this age group would be effective in reversing frailty in aged individuals. Theoretically, sustained hypovitaminosis D earlier in life could contribute to frailty in advanced ages. With

increasing age, thus decreased capability of coping with stressors, deteriorations caused by vitamin D insufficiency could become clinically relevant. In this case, supplementation should be initiated earlier in life in order to be beneficial. Further research is warranted to test this hypothesis.

PTH in older women

In this study, our primary aim was to describe the distribution of parathyroid hormone levels in older women and its changes during advancing age. Our secondary aim was to investigate the association between elevated PTH and all-cause mortality.

At baseline, most women had PTH concentrations within the normal range. Half of the women increased their PTH levels between ages 75 and 85, but only when these increases exceeded 50% from baseline was PTH elevated beyond the normal reference range.

In previous studies, even subtle elevations or elevations within the normal reference range have been associated with mortality^{175,176}. Although PTH elevated above normal was associated with mortality in our study, it was not an independent predictor but rather reflected a low vitamin D status and impaired kidney function. The latter appears to be the major up-regulator of PTH in the elderly¹⁷⁷.

Strengths and limitations

A particular strength of the OPRA-cohort study is the substantial number of women who were followed prospectively over a period of more than 15 years. Measurements and reevaluations after 5 and 10 years allowed us to investigate associations between vitamin D and various parameters in different time frames ranging from old to very old. It also allowed us to test consistency of identified associations over time. By combining vitamin D measurements from two different time points, we were able to more closely look at the “impact” of chronic or sustained hypovitaminosis D.

Another strength of this project is the fact, that the women included in the OPRA-cohort are all community-dwelling and mostly independently living. They could be considered as rather healthy with few comorbidities and disabilities. While we acknowledge that generalizations based on our study findings should be made cautiously, the advantage of studying a relatively healthy cohort is that it allows for a clearer investigation of vitamin D with less confounding from comorbidities.

Of course there are limitations to acknowledge as well. Firstly, the OPRA-cohort was primarily designed as a fracture-outcome study. Therefore, the power to detect other outcomes such as mortality and frailty may not always be sufficient, especially in the oldest age.

While we considered the use of vitamin D categories to be a more clinically relevant approach, it may have reduced the statistical power of some analyses. This is especially true for the fracture and mortality assessment where we investigated the importance of “chronic” hypovitaminosis D. In women in the upper or lower borderlines of a category, even slight deviations over time could lead to re-categorization and exclusion from further analyses although the actual vitamin D concentrations were more or less unchanged. (I.e.49 to 51 nmol/L).

Assessment of 25OHD is the most commonly used measurement of vitamin D and is the most reliable indicator of the total vitamin D supply. A limitation of currently available assays is their varying accuracy and reliability. In this project, 25OHD was assessed using high performance liquid chromatography mass spectrophotometry, a method that has been shown to be more accurate and consistent compared to antibody-based methods^{178,179}. Obviously, the assessment of active vitamin D (1,25-Dihydroxyvitamin D) would have allowed for a more sensitive analysis of the interplay between vitamin D and PTH, however, the high cost and associated technical issues prohibited this measurement.

Information on comorbidities was available at age 80 but not at age 75 and we did not have access to detailed information on the women’s medication. Furthermore, the cause of death was unknown.

General remarks and clinical implications

An important challenge is to understand whether a low vitamin D status is the cause or rather an indicator of impaired health in the older population. Results from randomized controlled trials and large meta-analyses regarding the effect of vitamin D on various diseases and mortality are far from consistent and the hypothesis that vitamin D insufficiency is the simply consequence of disease rather than its cause has been postulated¹⁸⁰. It is however reasonable to think that the connection between vitamin D and impaired health is a bi-directional. In older individuals, a decreased ability to synthesize vitamin D, a higher disease-burden with consecutively less time spent outdoors and eventually a higher prevalence of malabsorption, leads to lower vitamin D levels. This explains why an inadequate vitamin D status is frequently seen in frailer individuals and thus can be regarded an indicator of impaired general health. But based on our findings in the OPRA-cohort, a rather healthy cohort, low

vitamin D levels are associated with a higher risk of fractures for up to 10 years and, maybe most importantly, a higher risk of dying which was, based on available information, independent of underlying comorbidities. These results indicate that vitamin D indeed contributes to negative health outcomes in the aging population. Moreover, the fact, that observed associations remained significant over a long period, at least partially mitigates concerns about reverse causation. Vitamin D might play a minor role in the actual pathogenesis of various diseases and supplementation might therefore not be able to effectively prevent them. However, vitamin D could play an important role in the prognosis of various diseases commonly seen in the elderly.

The biggest controversy lies in the fact that we still lack consensus on how to define vitamin D sufficiency and insufficiency. Rather than focusing on the definitions, a desirable approach would be the identification of optimal vitamin D levels in older individuals. Based on our results, the optimal vitamin D concentration seems to be both target-specific as well as age-dependent. While levels above 50 nmol/L were associated with a significant risk reduction of hip and major osteoporotic fractures in women aged 75, levels above 75 nmol/L were needed in order to achieve the same benefits in the oldest old (age 85 years). While significantly lower mortality rates were seen in women who had high vitamin D concentrations above 75 nmol/L, frailty was not improved compared to women with vitamin D levels between 50 and 75 nmol/L. Overall, vitamin D concentrations above 75 nmol/L appear to be best suited for prevention of negative health outcomes in older women. A threshold of 75 nmol/L, which has been proposed by the American Endocrine Society¹⁸¹ and the International Osteoporosis Foundation³¹, would imply that the majority of older adults has suboptimal vitamin D levels. Supplementation at dosages between 1000 and 2000 IU per day would be needed in order to achieve such a high target. However, it is important to remember that current recommendations and guidelines regarding vitamin D supplementation are primarily based on the prevention of falls and fractures. Randomized controlled trials of high quality are needed in order to answer the question whether supplementation can prevent morbidity and mortality in the elderly.

In a clinical setting, vitamin D might be a useful marker in the risk assessment of future fractures and frailty. Although, the overall contribution of vitamin D to frailty seemed to be minor, low levels of 25OHD were associated with impaired muscle function and muscle strength. Since muscle weakness represents the most common first symptom of frailty, assessment of vitamin D could be helpful in identifying patients at risk for becoming frail. This would be of particular importance in patients with a fall history and thus risk of fractures and increasing frailty. It remains to be tested in randomized controlled trials whether early intervention with vitamin D supplements is able to reverse frailty, or at least, prevent its further progression.

Secondary hyperparathyroidism is common in aged individuals due to declining kidney function and inadequate vitamin D levels. Based on our findings, elevations of parathyroid hormone should always be evaluated in the context of kidney function and vitamin D status. Even elevations within the normal reference range were associated with lower 25OHD levels which could be seen as an argument for adjusting the upper threshold of the currently accepted normal PTH range downwards, especially in aged individuals. On the other hand, the most important up-regulator of PTH in the elderly seems to be the loss of kidney function, a well-known risk marker for mortality. The decline in eGFR was most pronounced in women who presented PTH concentrations well above the normal reference range. This fact could be used as justification for keeping the currently defined reference range.

Conclusions

In this thesis, investigating community-dwelling women, aged 75 and following them into their nineties, we found that low vitamin D levels were associated with a higher risk of fractures, a higher grade of frailty and an increased risk of dying.

In vitamin D sufficient women (>50 nmol/L) who remained sufficient over time, the incidence of major osteoporotic fractures was lower for up to a decade. However, considerably higher concentrations (>75 nmol/L) might be needed in the oldest old in order to achieve a significant risk reduction of hip fractures.

Sufficient vitamin D levels were also associated with a lower burden of frailty in all but the oldest old. Low vitamin D concentrations were correlated to impaired muscle strength and muscle function. In contrast to the observations on fractures, concentrations above 75 nmol/L were not found to be further beneficial in regards to frailty.

High vitamin D levels were however associated with a lower risk of dying and the observed association was, at least partially, independent of health status and fracture-related frailty.

Despite substantial increases of PTH over time, the majority of women maintained levels within the normal reference range. PTH was closely linked to the women's vitamin D status and kidney function and elevations of PTH were not found to be independent predictors of frailty or mortality.

In summary, maintaining sufficient vitamin D levels appears to be beneficial for healthy ageing, both from a musculoskeletal perspective as well as in terms of general health. These findings highlight the importance of identifying and supplementing older individuals at high risk of hypovitaminosis D.

Future perspectives

In recent years, there has been a profound interest in vitamin D both within the research community as well as in the general population. Substantial variations in the daily dietary intake of vitamin D can be seen across European countries¹⁸². The lowest intakes in individuals above the age of 64 years were seen in Spain (28 IU/d) and the highest in Nordic countries (Sweden: 240-280 IU/d). According to a survey from 2009¹⁸³, 28% of men and 41% of women in Sweden were taking dietary supplements. The use of vitamin D supplements has increased enormously in the past decade. In 2009, the amount spent on these supplements had increased tenfold within 10 years¹⁸⁴ and the market is expected to rise further in the future.

Our knowledge of vitamin D and its functions in the human body has greatly improved thanks to a large amount of research, in particular observational studies that found associations even outside the musculoskeletal system. Given the ubiquitous presence of vitamin D receptors in humans, it is not too surprising that vitamin D may play a role in various different pathways determining our health. However, being a contributing factor may not always mean that it is of major significance in a clinical setting.

This urges the necessity of well-coordinated intervention trials. Such research should focus on older individuals with verified vitamin D insufficiency or deficiency representing an important risk group. Conducting trials with different dosages of vitamin D and reassessments to ensure target levels are reached would eventually allow us to identify threshold levels needed in order to maintain musculoskeletal health as well as providing valuable insights to whether vitamin D supplementation is able to improve various aspects of health that are compromised as a result of ageing.

Hopefully, this thesis has contributed to a better understanding of the importance of adequate vitamin D levels in older women in regards to musculoskeletal health. Thanks to the substantial number of women and the amount of data available, further research can be conducted in this cohort. It would be of great interest to explore the association between vitamin D and sarcopenia in more detail as well as the interaction with fibroblast growth factor 23 (FGF-23), an important regulator of vitamin D homeostasis.

My initial assumption was that vitamin D would be increasingly important as the women got older. However, it turned out that it was difficult to establish associations between vitamin D and other variables once the women had reached very old age. This could be due to the limited number of women who were still alive at the end of the study. Furthermore, the increasing amount of comorbidities could become a much more important determinant of general health substantially decreasing the impact of vitamin D. But we could also speculate that inadequate vitamin D levels should be corrected earlier in life in order to have beneficial effects on fracture, frailty and mortality. It would be tempting to conduct a new observational study similar to the OPRA-cohort but resetting the baseline to 65 years in order to see whether the associations we found were linked to the current vitamin D status or rather consequences of inadequate vitamin D levels earlier in life.

Ethical Considerations

The study was performed according to the principles of the Helsinki declaration and was approved by the Regional Ethical Review Board in Lund. Written informed consent was obtained from all women and participation was entirely voluntary.

The risk of harm was considered to be low due to the observational character of this study. However, dealing with older individuals and following them over a long time raises some ethical issues.

Respect and Justice

With advancing age and loss of physical functioning, it becomes increasingly important to respect the individual's autonomy and to protect those with impaired autonomy from harm and possible exploitation. Justice requires a fair balance between benefits and burdens of participation. In this study, all individuals were treated with respect and inclusion of participants was not limited on grounds of ethnicity, disabilities or religious beliefs. Unwillingness to participate, illness and death were the only exclusion criteria.

Study Design and Conflict of Interest

The observational study design was considered to impose minimal risk of harm to participants. The investigators and co-investigators had no conflict of interest with regards to treatment or participation in the study.

Protocol

The study was conducted according to a written protocol including the aim of the study, the data and number of participants needed and the statistical procedures required.

Hawthorne Effect and Selection Bias

The Hawthorne effect¹⁸⁵, a process of subjects changing their behavior due to the fact that they are observed, cannot be fully excluded. Since the OPRA study was primarily a fracture outcome study and due to the fact that the observation took place over a long time period, participants may have been inclined to adapt a more active lifestyle during the observational period. However, given the high age of participants, the size of this effect would presumably be rather small.

Selection bias has to be considered given the fact that healthier individuals were more likely to participate and were less likely to be lost during follow up. Any generalizations of these study results should therefore be made cautiously.

Appendix I

Important randomized controlled trials investigating the fracture preventive effect of vitamin D supplementation

In this appendix section, a summary of important randomized controlled trials investigating the effect of vitamin D supplementation on fractures is provided. As stated before, results from previous trials are inconsistent. In general, trials performed in older and institutionalized participants with a presumably higher prevalence of hypovitaminosis D, as well as trials using dosages between 700 and 800 IU per day were more likely to show significant treatment effects. Lower as well as very high dosages given annually appeared to be ineffective or even increased the risk of fracture (Table 9).

Table 9. Summary of Vitamin D and fracture RCTs

This table summarizes important randomized controlled trials that investigated the effect of vitamin D supplementation on fracture risk. Studies are highlighted in blue (significant risk reduction) and red (no significant effect); * = meta-analysis

	Year	Characteristics	Main findings
Chapuy ¹⁸⁶	1992	3270 healthy ambulatory women, aged 84y, randomized to 18 months of daily tricalcium phosphate (containing 1.2 g of elemental calcium) and 20 µg (800 IU) of vitamin D3 or placebo	Supplementation with vitamin D3 and calcium reduced the risk of hip fractures by 43% and nonvertebral fractures by 32%.
Heikinheimo ¹⁸⁷	1992	Ergocalciferol (150,000-300,000 IU) was given annually to two series of aged subjects: first to 199 (45 male) of 479 subjects (110 male) aged 85+ y who were living in their own home, and second to 142 (29 male) of 320 (58 male) institutionalized subjects aged 75-84y. Injections were given 2-5 times to each participant.	After 1 year: The risk of all fractures was reduced by 24%. Significant risk reduction only in women and of fractures in the upper extremities
Dawson-Hughes ¹⁸⁸	1997	176 men and 213 women 65 years of age or older who were living at home. They received either 500 mg of calcium plus 700 IU of vitamin D3 (cholecalciferol) per day or placebo for 3 years.	Moderate risk reduction of nonvertebral fractures, n=26 in placebo group 11 in calcium-vitamin D group
Trivedi ¹³²	2003	2686 people (2037 men and 649 women) aged 65-85 years living in the general community, randomized to 100 000 IU of oral cholecalciferol or placebo every four months for 5 years.	After 5 years, the relative risk was reduced by 22% for any first fracture and by 33% for nonvertebral fractures
Larsen ¹⁸⁹	2004	9605 community-dwelling residents aged 66+ years. A prevention program of a daily supplement of 1000 mg of elemental calcium as calcium carbonate and 400 IU of vitamin D3 to was offered to 4957 participants (participation=50%). Another program with evaluation and suggestions for the improvement of the domestic environment was offered to 5063 participants (participation=46%). Duration=3 years	Relative risk reduction of nonvertebral fractures was 16%.
Grant ¹⁹⁰	2005	Factorial-design trial, 5292 people aged 70 years or older (85% women) who were mobile before developing a low-trauma fracture were randomly assigned 800 IU daily oral vitamin D3, 1000 mg calcium, oral vitamin D3 (800 IU per day) combined with calcium (1000 mg per day), or placebo and followed for 24-62 months.	Oral supplementation with calcium and vitamin D3, either alone or in combination, did not significantly decrease the risk of further fractures.

Table 9. Summary of Vitamin D and fracture RCTs (continued)

	Year	Characteristics	Main findings
Jackson ¹⁹¹	2006	36,282 postmenopausal women, 50 to 79 years of age, who were already enrolled in a Women's Health Initiative (WHI) clinical trial were randomly assigned to receive 1000 mg of elemental calcium as calcium carbonate with 400 IU of vitamin D3 daily or placebo. Average follow-up =7 years.	Calcium with vitamin D supplementation resulted in a small but significant improvement in hip bone density but did not significantly reduce hip fracture
Porthouse ¹⁹²	2005	3314 women aged 70 and over with one or more risk factors for hip fracture: any previous fracture, low body weight (< 58 kg), smoker, family history of hip fracture, or fair or poor self reported health. Daily oral supplementation using 1000 mg calcium with 800 IU cholecalciferol and information leaflet on dietary calcium intake and prevention of falls, or leaflet only (control group). Median follow-up=25 months.	Calcium and vitamin D supplementation did not reduce the risk of clinical fractures in women with one or more risk factors for hip fracture.
Lyons ¹⁹³	2006	Pragmatic double blind randomised controlled trial of 3 years duration. 3,440 people (2,624 women and 816 men) living in residential or care home were investigated and assigned to four-monthly oral supplementation with 100,000 IU ergocalciferol or placebo	Supplementation with four-monthly 100,000 IU of oral vitamin D2 did not reduce fracture incidence.
Smith ¹⁹⁴	2007	9440 people (4354 men and 5086 women) aged 75 yrs and over were recruited from general practice registers and assigned to 300,000 IU intramuscular ergocalciferol injection or matching placebo every autumn over 3 years. Primary outcome measure was all non-vertebral fracture.	No fracture-preventive effect of vitamin D supplementation
Sanders ¹⁹⁵	2010	2256 community-dwelling women, aged 70 years or older, considered to be at high risk of fracture were randomly assigned to receive a single annual dose of 500 000 IU cholecalciferol orally or placebo each autumn to winter for 3 to 5 years.	High-dose cholecalciferol resulted in an increased risk of falls and fractures.
Bischoff-Ferrari ¹⁹⁶ *	2005	Five RCTs for hip fracture (n = 9294) and 7 RCTs for nonvertebral fracture risk (n = 9820)	Cholecalciferol at a dose of 700 to 800 IU/d reduced the relative risk of hip fracture by 26% and any nonvertebral fracture by 23% compared to calcium or placebo.

Table 9. Summary of Vitamin D and fracture RCTs (continued II)

	Year	Characteristics	Main findings
Bischoff-Ferrari ¹⁹⁷ *	2009	12 double-blind randomized controlled trials (RCTs) for nonvertebral fractures (n = 42 279) and 8 RCTs for hip fractures (n = 40 886) comparing oral vitamin D, with or without calcium, with calcium or placebo.	Cholecalciferol at doses >400 IU reduced nonvertebral fractures in community-dwelling individuals (-29%) and institutionalized older individuals (-15%), and its effect was independent of additional calcium supplementation.
DIPART group ¹⁹⁸ *	2010	Seven major randomized trials of vitamin D with calcium or vitamin D alone with a total of 68 517 participants (mean age 69.9 years, range 47-107 years, 14.7% men).	Vitamin D given alone in doses of 400-800 IU was not effective in preventing fractures. Calcium and vitamin D given together was found to reduce hip fractures and total fractures, irrespective of age, sex, or previous fractures
Avenell ¹⁹⁹ *	2014	53 trials with a total of 91,791 participants. 31 trials, with sample sizes ranging from 70 to 36,282 participants, examined vitamin D with or without calcium in the prevention of fractures in community, nursing home or hospital inpatient populations.	Vitamin D alone was not found to prevent fractures in the doses and formulations tested so far in older people. Supplementation of vitamin D and calcium was associated with a small but significant risk reduction of hip or any type of fracture.

Appendix II

In this appendix, additional data is presented that, although not part of the manuscripts constituting this thesis, may contribute towards answering additional questions surrounding the role of vitamin D in bone metabolism.

Vitamin D, PTH and bone loss

In general, associations between vitamin D, PTH and bone loss were weak and not consistent over time. Although not statistically significant, we observed a trend towards higher bone loss in women with insufficient vitamin D and elevated PTH levels compared to those who maintained 25OHD and PTH concentrations within the sufficient or normal range. This trend was seen at all measured sites (trochanter, total hip, femoral neck) except for the lumbar spine (data not shown) where bone density seemed to increase between ages 75 and 80, possibly explained by increased vertebral deformities. The fact that low vitamin D levels were associated with increased fracture risk despite the fact that bone loss was not significantly increased was somewhat surprising. However, we can speculate that the advanced age of the women could have influenced the results. As seen in Figure 4 (page 23), bone loss is most pronounced during the first 10-15 post-menopausal years and plateaus later in life due to altered biochemical sensing of mechanical loading by osteocytes⁵⁴.

To further investigate bone loss in women with sustained hypovitaminosis D, bone loss rates were calculated between ages 75 and 80 years in women who had low (<50 nmol/L), intermediate (50-75 nmol/L) and high (>75 nmol/L) 25OHD levels at both measurement times. Total bone loss was assessed at the trochanter, total hip and femoral neck.

As seen in Figure 17, bone loss seemed to be higher in women with sustained hypovitaminosis D, although significance was only reached at the trochanter.

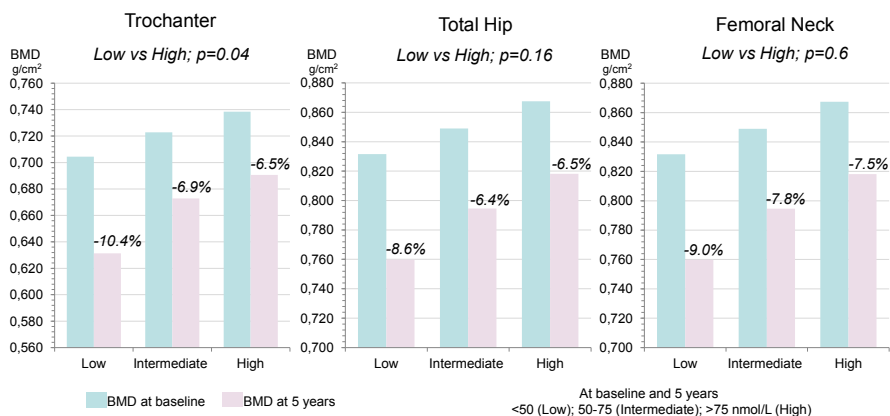


Figure 17. Vitamin D and bone loss between ages 75 and 80 years

This figure shows the total bone loss at the trochanter, total hip and femoral neck between ages 75 and 80 years in women with continuously low, intermediate and high levels of 25OHD.

To investigate the association between PTH and bone loss, we assessed the total loss of bone between ages 75 and 80 years in women who had PTH levels within the normal reference range (1.6 – 6.9 pmol/L) compared to women where PTH concentrations were elevated above normal (>6.9 pmol/L) at both ages 75 and 80 years. No association between PTH and bone loss was found as shown in Figure 18.

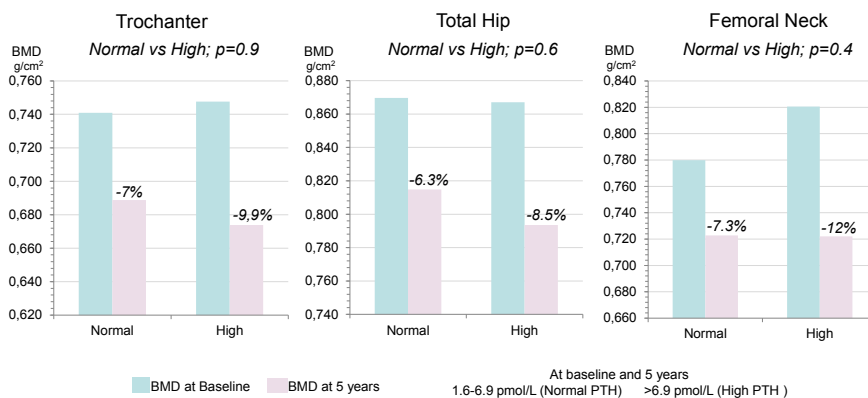


Figure 18. PTH and bone loss between ages 75 and 80 years

This figure shows the total bone loss at the trochanter, total hip and femoral neck between ages 75 and 80 years in women with PTH levels within the normal range and women with elevated PTH at both time points.

Data on vitamin D, PTH and bone loss has been presented as abstract²⁰⁰, but due to difficulties in stratifying bone loss and interpreting the data, we chose to abstain from publishing these results in any scientific journal.

Elevated PTH and hip fractures

No association between elevated PTH at baseline and hip fractures was found, neither unadjusted nor adjusted for 25OHD and kidney function. The small number of women with suspected primary (n=23) or secondary hyperparathyroidism (n=4) at baseline was a limiting factor making any further analyses impossible.

Hip fracture rates in women with PTH within the normal range and women with elevated PTH can be seen in Table 10.

Table 10. Hip fracture rates in women with normal and elevated PTH at baseline

This table shows the number of women who sustained a hip fracture during follow-up (until year 2006) based on whether PTH was normal or elevated above the normal reference range. p-value compares hip fracture rates in women with normal and elevated PTH, Values are numbers and (percentage).

All women	Hip fracture (until 2006)	No hip fracture	p*
PTH 1.6-6.9 pmol/L (n=877)	97 (11)	780 (89)	0.455
PTH >6.9 pmol/L (n=109)	9 (8)	100 (92)	

*comparing women with normal to women with elevated PTH at age 75

Seasonal variation of vitamin D

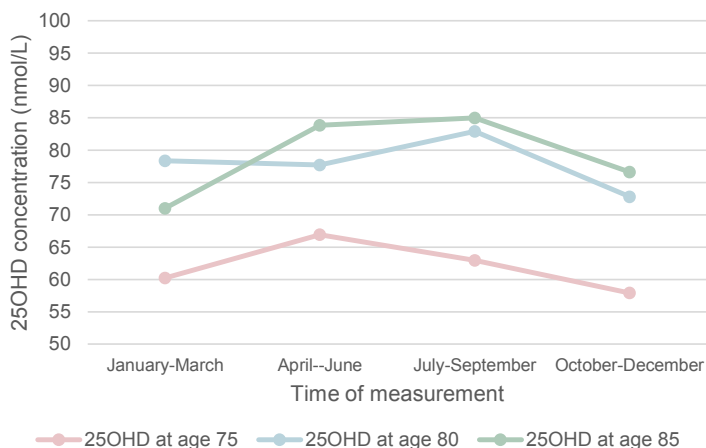


Figure 19. Seasonal variation of vitamin D

The mean 25OHD concentrations of the OPRA cohort were 62 (age 75), 78 (age 80) and 79 nmol/L (age 85). Concentrations varied dependent on the time of measurement throughout the year. At age 75, the lowest 25OHD concentrations

were found between October and December (mean 58 nmol/L) and the highest between April and June (67 nmol/L; $p < 0.001$). At ages 80 and 85, 25OHD was highest during summer between July and September (83 and 85 nmol/L) and lowest between October and April (73 and 71 nmol/L) ($p = 0.003$ and $p = 0.001$) (Figure 19.).

Although statistically different, the clinical relevance of these seasonal variations is debatable with a maximum difference between the lowest and highest values of only 14 nmol/L. Previous research has shown that occasional UVB exposure during summer months is able to increase vitamin D by up to 40 nmol/L depending on age, body mass and pigmentation^{201,202}.

Research conducted in healthy men and women from Gothenburg indicates that vitamin D supply from sun exposure might be insufficient except for a few months during summer²⁰³. We therefore additionally investigated the seasonal variation of vitamin D based on months with presumably insufficient sun exposure (October-April) compared to months where regular outdoor activities would be able to provide adequate vitamin D supply. (Figure 20.)

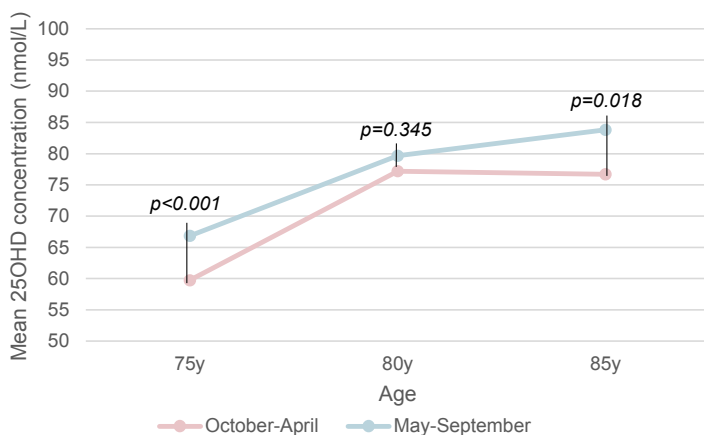


Figure 20. Seasonal variation of 25OHD based on sufficient or insufficient sun exposure

Interestingly, the seasonal variation of 25OHD was not consistent over time. At ages 75 and 85, the mean concentrations of 25OHD samples taken between October and April were lower compared to those taken during summer months (60 vs 67 nmol/L, $p < 0.001$ and 77 vs 84 nmol/L, $p = 0.018$). At age 80 however, no significant difference in 25OHD was found.

However, the proportion of women who were vitamin D insufficient remained relatively stable throughout the year except for measurements at age 75 where a significant increase was seen between October and April. (Table 11.)

Table 11. Proportion of women with 25OHD < 50nmol/L between May-September and October-April

Age (years)	May-September (%)	October-April (%)	<i>p</i>
75	22	30	<i>0.034</i>
80	16	16	<i>0.939</i>
85	9	13	<i>0.387</i>

The findings in this thesis are presented unadjusted for the time of measurement. However, additional adjustment for seasonal variations (based on month, quarter or available sun exposure) did not alter the original results in any significant way.

Svensk sammanfattning

Bakgrund till avhandlingen

D-vitamin har en viktig funktion i regleringen av kalkbalansen i kroppen.

Den viktigaste källan till D-vitamin är solen som stimulerar produktionen i huden. Vissa livsmedel innehåller D-vitamin (t.ex lax, sill, ägg,..) eller berikas med D-vitamin så som mjölkprodukter och margarin.

Uttalad brist på D-vitamin kan orsaka sjukdomen rakit som även kallas osteomalaci hos vuxna. Denna karakteriseras av muskelsvaghet och muskelvärk och uppvisar en tydlig urkalkning av skelettet. D-vitamin spelar även en viktig roll i regleringen av benomsättningen och otillräckliga nivåer leder till en obalans mellan celler som bygger upp och celler som bryter ner skelettet. Konsekvensen är utvecklingen av osteoporos, en sjukdom som kännetecknas av ett mer ihåligt och mindre hållfast skelett.

I tidigare forskning har man sett kopplingar mellan D-vitaminbrist och risken för fall och frakturer. Under senare åren har dock även så kallade extraskelletala effekter av D-vitamin uppmärksammats. Observationsstudier har visat samband mellan D-vitaminbrist och exempelvis hjärt-kärlsjukdomar, cancer och diabetes. Randomiserade, placebo-kontrollerade interventionsstudier har dock i många fall inte kunnat verifiera dessa samband.

Parathormon (PTH) är ett hormon som tillsammans med D-vitamin reglerar kalkomsättningen. Stigande PTH värden är relativt vanligt förekommande hos äldre och beror då framförallt på för låga D-vitamin nivåer eller nedsatt njurfunktion. Förhöjda PTH värden bidrar till osteoporosutveckling och har i tidigare forskning kopplats till högre dödlighet.

En grupp med hög risk för D-vitaminbrist är äldre människor. I många fall är utomhusvistelser otillräckliga samtidigt som hudens produktion minskar med åldern. Ett nedsatt upptag från tarmen och otillräcklig nutrition kan vara bidragande orsaker.

Materialet till denna avhandling är 75-åriga kvinnor från Malmö. Följande aspekter undersöktes:

- Sambandet mellan långvarigt D-vitaminbrist och risken för frakturer
- Sambandet mellan D-vitaminbrist och dödlighet
- Sambandet mellan D-vitaminbrist och ”allmän skörhet” (frailty)
- Ändringar av parathormon (PTH) nivåerna över tid och sambandet mellan förhöjda värden och skörhet och dödlighet

OPRA-kohort

1044 75-åriga kvinnor från Malmö inkluderades mellan 1996 och 1999. Uppföljningar ägde rum efter 5 och 10 år. Vid besöken genomfördes bland annat bentäthetsmätningar, muskelfunktionstester och analyser av blodprover. Frakturer och information om dödsfall registrerades kontinuerligt fram till oktober 2012.

Resultat

Kvinnor som hade kronisk D-vitaminbrist (<50 nmol/L) under 5 år uppvisade en signifikant högre risk att få en osteoporosrelaterad fraktur inom 10 år. Låga D-vitamin nivåer var även kopplade till en ökad grad av skörhet och dessa kvinnor hade en högre risk att dö inom 5 och 10 år. Kopplingen mellan D-vitamin och dödlighet var åtminstone delvis oberoende av andra sjukdomar, frakturer och livsstilsfaktorer. Ungefär hälften av kvinnorna ökade sina PTH värden under uppföljningstiden vilket var kopplat till lägre D-vitamin och sämre njurfunktion. Ett förhöjt PTH var dock inte en oberoende riskfaktor för skörhet och dödlighet.

Anmärkningar

D-vitaminbrist är vanligt förekommande hos äldre individer. Resultaten i denna avhandling visar på betydelsen av D-vitaminbrist i den äldre befolkningen genom att påvisar en tydlig koppling mellan låga D-vitamin nivåer och risken för frakturer och död. Det ligger dock i observationsstudiernas natur att inte kunna bevisa det kausala sambandet. Välgjorda interventionsstudier med fokus på äldre behövs för att kunna undersöka huruvida substitutionsbehandling med D-vitamin kan minska frakturrisken och förbättra överlevnaden.

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Vitamin D In Older Women

Vitamin D is essential for calcium and phosphate homeostasis and plays an important role for the musculoskeletal system. Severe vitamin D deficiency can lead to osteomalacia or rickets, a disease characterized by mineralization deficits in the skeleton, muscle pain and weakness. During the 19th century, exposure to sunlight was found to be an effective cure of rickets.

Almost 200 years later, we know that most of the actions of vitamin D are carried out through interaction with vitamin D receptors which can be found almost ubiquitously in the human body. At least in theory, the actions of vitamin D should go beyond the "classical" musculoskeletal and calcium-regulating functions of vitamin D. In fact, associations have been found between low vitamin D levels and negative health outcomes in various organ tissues and systems. However, results from randomized controlled trials are far from consistent.

Vitamin D levels below 25 nmol/L are commonly accepted as vitamin D deficient. We are however still lacking a consensus definition of vitamin D insufficiency as well as clear threshold values of what to regard as optimal. Regardless of the definition, older individuals are at high risk of developing hypovitaminosis D.

In this thesis, we used data from 1044 community-dwelling women, aged 75 and followed into their nineties, to investigate the association between vitamin D insufficiency and fractures, frailty and mortality. Additionally, we described the distribution of parathyroid hormone in relation to vitamin D and kidney function and its association to frailty and mortality.

In summary, low vitamin D levels were associated with a higher risk of fractures, a higher grade of frailty and an increased risk of dying. The association between vitamin D and fractures was even more pronounced in women who had chronic or sustained vitamin D insufficiency. The increase in mortality was, at least in part, independent of comorbidities and fractures. Elevated parathyroid hormone was not found to be an independent predictor of frailty or mortality.

