Vitamin D and Functional Performance in Ageing

Christian Oudshoorn

Colophon
Design, cover & graphics Ridderprint
Printed by Ridderprint
ISBN 978-94-6299-411-6

Cover photo by: J. Tepper, Pyongyang, 2008

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Vitamine D en functionaliteit bij veroudering

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de
rector magnificus
Prof.dr. H.A.P. Pols
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatsvinden op
1 november 2016 om 15:30 uur
door
Christian Oudshoorn
geboren te Leiden.

Erasmus University Rotterdam

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Section 1

General introduction

GENERAL INTRODUCTION

FUNCTIONAL PERFORMANCE IN AGEING

Functional performance declines with ageing. During the ageing process all tissues and organ systems decline in function, resulting in a decreased functional reserve and a state of increased vulnerability. Key component in the functional decline in older persons is a decline in muscle mass and strength (sarcopenia). Sarcopenia is associated with various adverse health outcomes such as an increased fall- and fracture-risk, poor quality of life and increased mortality risk[1]. In this regard, vitamin D seems of particular interest because of the pleiotropic function of this hormone, which include effects on muscle, bone, neurons and vascular tissue. In addition, specific age-related changes occurring in the vitamin D and calcium homeostasis lead to specific risks and deficiencies in older individuals, which contribute to the functional decline.

VITAMIN D

Vitamin D is a fat soluble, seco-steroid hormone. Regulation of calcium and phosphate homeostasis is generally regarded as the traditional role of this hormone. The main sources of vitamin D are cutaneous production, dietary intake and supplement use. After exposure to sunlight, the 7-dehydrocholesterol in the skin is converted to vitamin D_3 under the influence of UV-B (290-315nm) [2]. The amount of vitamin D synthesized in the skin depends on environmental factors such as geographic latitude, season, and cloud coverage, and personal characteristics such as skin pigmentation, age and time spent outdoors. Further factors influencing vitamin D production in the skin are clothing (especially UV-protective clothing is now widely available) and use of sunscreen[3]. Some dietary products contain natural vitamin D (either vitamin D_2 (ergocalciferol) or D_3 (cholecalciferol)), such as fatty fish and dairy products. It is estimated that sunlight supplies 90% of the vitamin D need, whereas dietary intake contributes just 10% of the vitamin D need[4]. The precursors vitamin D_3 or D_2 are metabolized in the liver to 25-hydroxyvitamin D_3 ($25OHD_3$) by the enzyme 25-hydroxy-

lase. $25OHD_3$ is the main circulating vitamin D metabolite with a half life of 2 to 3 weeks. This is the why serum $25OHD_3$ is regarded to best reflect a person's vitamin D status[5].

Next, $25OHD_3$ is converted into $1,25OH_2D_3$, the most potent vitamin D metabolite, by the enzyme 1α-hydroxylase (CYP27B1). Most enzymatic 1α-hydroxylase activity is located in the kidney, making this the most important organ for activation of the vitamin D metabolites. This 1α-hydroxylase process is tightly regulated by a feedback mechanism including parathyroid hormone (PTH) concentration, serum calcium concentration and fibroblast-growth-factor 23 (FGF-23) expression[6]. The 1α-hydroxylase enzyme is also expressed by other various tissues, such as osteoblasts, dendritic cells and keratinocytes[7, 8]. This enables peripheral (extra-renal) $1,25OH_2D_3$ formation and thus paracrine and autocrine effects. The majority (85-90%) of the circulating $25OHD_3$ and $1,25OH_2D_3$ is tightly bound to the vitamin D binding protein (DBP). In addition, about 10-15% of the metabolites is bound to albumin, leaving a small fraction of less than 1% as free, unbound vitamin D metabolites[9]. It is thought that especially the unbound metabolites exert a biological effect.

The vitamin D metabolites exert their effects via the vitamin D receptor (VDR) and this includes both genomic and non-genomic effects. Genomic effects of vitamin D are mediated by gene transcription and subsequent de novo protein synthesis. This process is initiated by the binding of the vitamin D metabolites to the high affinity nuclear VDR. The nuclear VDR is expressed in almost all tissues and cell types[10, 11]. The vitamin D receptor gene (VDR) is located on chromosome 12 (12q13.1). After binding of $1,25OH_2D_3$ to the nuclear VDR it forms a heterodimer with the retinoid-X receptor (RXR) protein. This heterodimer binds to a VDR-specific response element in the DNA and acts as a regulator of gene transcription[12]. Non-genomic functions of vitamin D are generally rapid responses (minutes to hours). These responses are mediated by binding of vitamin D metabolites to the plasma membrane associated VDR[13]. The subsequent effects take place in the cytoplasm of the cell rather than in the nucleus. Plasma membrane associated VDR effects include modulation of intracellular calcium levels and activation of intracellular signaling cascades. Non-genomic actions have especially been well-described in muscle tissue and in osteoblasts[8, 14].

AGEING AND VITAMIN D

Ageing is associated with an increased risk for vitamin D deficiency. The estimated prevalence of vitamin D deficiency in older persons ranges from 40-100% in community dwelling elderly men and women in both the US and Europe, depending on the definition and cut-off values used[15]. In the Netherlands, a persons with a serum $25OHD_3$ level < 50nmol/l is generally considered vitamin D deficient. The high prevalence of vitamin D deficiency among older persons has several causes. The most important cause is probably a marked decrease in cutaneous vitamin D production capacity. Earlier studies in the 1980's demonstrated a more than twofold decrease in cutaneous vitamin D_3 production capacity in older individuals as compared to young individuals which is due to an age-dependent decrease in epidermal pro-vitamin D_3 (7-dehydrocholesterol) concentration[16]. Other contributing causes to the high prevalence of vitamin D deficiency in older individuals are discussed in more detail in chapter 2.1

Ageing is also thought to be associated with a decreased efficiency of the vitamin D endocrine system and calcium homeostasis. For example, VDR expression in certain tissues has been reported to decrease with ageing, which could lead to a certain vitamin D resistance in older individuals[17, 18]. Other reported effects of ageing that could decrease the efficacy of the vitamin D endocrine system and calcium homeostasis are an age dependent decrease in both renal and intestinal calcium channel expression and the increased prevalence of renal insufficiency in older persons which leads to a decrease in renal 1α-hydroxylase activity[18, 19]. The precise effects of ageing on the vitamin D endocrine system and calcium homeostasis are also discussed in more detail in chapter 2.1.

The high prevalence of vitamin D deficiency in old age, its causes, and effects have been known for many years now. In recent years vitamin D related research has generated much interest. This has resulted in guidelines, implemented care plans and protocols on the screening for, and treatment of, vitamin D deficiency in the Netherlands[20-22]. However, despite these efforts, still many older persons remain untreated. It is unclear why, despite all these efforts, vitamin D deficiency is still so prevalent among older persons. A hypothesis could be that currently most studies, protocols and care plans focus on doctor-driven initiatives and interventions for the diagnosis and treatment of vitamin D deficiency. Patient

related factors are not, or scarcely studied. For example, no study examined the knowledge on vitamin D among older persons and if patient-knowledge on vitamin D is associated with vitamin D status or health behavior. Therefore we performed a study on knowledge of vitamin D among older persons, and studied the association with a person's vitamin D status. This issue is discussed in more detail in chapter 2.2.

VITAMIN D AND FUNCTIONAL PERFORMANCE

Vitamin D and muscle function

Mobility and muscle function decline with ageing[23]. A key contributing factor in this process is the decrease in muscle mass and muscle strength, also named sarcopenia. Sarcopenia is a common condition in older adults. From approximately 40 years of age, a progressive loss of muscle mass occurs. Until the age of 70 years this loss is estimated at about 8% per decade and increases to about 15% per decade in individuals over the age of 70. As a result, muscle strength declines with ageing[24, 25]. The process of sarcopenia is generally thought to be multifactorial, influenced by environmental factors, disease triggers, inflammatory pathway activation, and hormonal factors[26]. Vitamin D is, since long, considered important for muscle mass and muscle strength. The first observations between vitamin D deficiency and (neuro)muscular dysfunction date back to the fifties and sixties[27]. The classical symptom of vitamin D associated myopathy is proximal muscle weakness. Other common symptoms include gait disturbances ("waddling gait"), and uniform generalized muscle wasting[28]. With the identification of VDR expression in skeletal muscle cells, muscle tissue was recognized as a direct target tissue for vitamin D[29]. Histological studies in human skeletal muscles demonstrate that vitamin D deficiency is associated with type II, fast twitch, muscle fiber atrophy[30]. Other observed changes are enlarged interfibrillary spaces, increase in fat infiltration, fibrosis and accumulation of glycogen granules[31]. Additionally, several rapid, non-transcriptional effects of vitamin D on skeletal muscle cells have also been reported, suggesting that the membrane bound VDR is also expressed by skeletal muscle cells[13]. Considering the significance of vitamin D for muscle function and mobility, it is important to recognize that a broad range of neurological effects of vitamin

D have been reported. Specific neurological effects of vitamin D, both on the central and peripheral nervous system, could also influence mobility. These neurological effects of vitamin D will be discussed in greater detail below.

In recent years many studies have been published that examine the association between vitamin D status and muscle function[32]. While some observational studies found no association, the majority of the observation studies did demonstrate an association between vitamin D status and muscle function[33]. For example, in an observational study performed by Bischoff-Ferrari et al., higher serum $25OHD_3$ levels were associated with better lower-extremity function in persons aged ≥ 60 years. In this study that included 4100 individuals, subjects with serum $25OHD_3$ levels between 22.5 and 40nmol/l, had better lower extremity function compared to subjects with lower levels. This trend between lower extremity function and serum $25OHD_3$ levels was seen up to serum levels of 94nmol/l[34]. In the prospective Longitudinal Aging Study Amsterdam, lower serum $25OHD_3$ levels were associated with a decrease in grip strength and appendicular muscle mass in older men and women during a three year follow up period[35].

While the results of observational studies that examine the association between muscle function and vitamin D status are mostly consistent, the results of intervention trials that study the effect of vitamin D supplementation on muscle function are more inconsistent. While some studies reported an improvement of muscle function with vitamin D supplementation, other studies found no effect of vitamin D supplementation on muscle function or strength[33]. Causes for the inconsistency of the trial results could be a difference in severity of vitamin D deficiency, differences in supplementation products or dosages, variation in co-morbidity or functional performance and variation in trial endpoints.

To date, the optimal serum $25OHD_3$ level for muscle function is not known. Several reports suggest that $25OHD_3$ levels of up to 90nmol/l could be needed for optimal muscle function[36]. However, these serum levels are not reached in many trials as many studies used low dose vitamin D supplementation. Therefore it could be hypothesized that high dose supplementation is preferable for the treatment of vitamin D associated myopathy and that high dose supplementation results in a marked increase in muscle performance. The effect of high dose supplementation is discussed in more detail in chapter 3.2.

Vitamin D and cardiovascular function

The incidence of cardiovascular events increases with ageing and coincides with the rising prevalence of vitamin D deficiency in older individuals. In recent years, many studies reported on associations between vitamin D deficiency and a variety of cardiovascular risk factors and disease[37]. Earlier studies demonstrated that the prevalence of vitamin D deficiency is high among individuals with cardiovascular disease (CVS). In the National Health and nutrition Examination Survey (NHANES), 68-97% of the participants had serum $25OHD_3$ levels < 75nmol/l[38]. The assumption that vitamin D status modulates cardiovascular risk is partly based on the fact that the enzyme 1α-hydroxylase is expressed in vascular smooth muscle cells (VSMC), endothelial cells, macrophages and dendritic cells, thereby enabling local $1,25OH_2D_3$ production. Furthermore, the VDR is widely expressed by various tissues and cells of the cardiovascular system including cardiac muscle cells, endothelium and vascular smooth muscle cells[37].

In a study by Li et al., vitamin D has been shown to be a negative regulator of the rennin-angiotensin-aldosterone system (RAAS)[39]. In another study, low vitamin D levels were associated with an increased activity of RAAS[40]. In VDR knockout models there is an increase in the expression of rennin and consequently of angiotensin II production, resulting in hypertension and cardiac hypertrophy[39]. In 1α-hydroxylase knockout models, mice have elevated levels of rennin, hypertension and cardiac hypertrophy. This is reversed by treatment with $1,25OH_2D_3$[41]. Furthermore, vitamin D is thought to play a pathophysiological role in the development of both types 1 and 2 diabetes mellitus[42]. Additionally, immune and inflammatory cells play an important role in CVD and the development of atherosclerosis[37]. Various cells involved in the immune system such as macrophages, dendritic cells and activated T cells express the VDR. In general the effects of vitamin D are thought to by anti-inflammatory. For example, vitamin D down-regulates pro-inflammatory cytokines such as interleukin-1 (IL-1), IL-6, IL17, IL-23, tumor necrosis factor-α and interferon-γ, while anti-inflammatory cytokines such as IL-4 and IL-10 are up-regulated[43, 44].

While cross sectional and observational data suggest an association between vitamin D status and cardiovascular heath, most intervention studies failed to demonstrate an effect. So, to date, the importance for vitamin D for cardio-

vascular health is still subject of debate. In this thesis we study if all arteries, both central and peripheral, are affected in the same way by states of vitamin D deficiency in a cohort of older people. This is discussed in chapter 4.1.

Vitamin D and neurological function

Several years ago, vitamin D was named a forgotten neurosteroid[45]. Like other steroid hormones, vitamin D metabolites have been shown to cross the blood-brain barrier and are present in the cerebrospinal fluid[46]. In addition, the enzyme 1α-hydroxylase is expressed throughout in the brain enabling $1,25(OH)_2D_3$ formation locally in the central nervous system (CNS). In 2005 the VDR expression in the human brain was extensively mapped by Eyles et al. who demonstrated that the VDR expression, in addition to the enzyme 1α-hydroxylase, is widely distributed throughout the adult human brain[47]. VDR expression is especially prominent in the large (presumably dopaminergic) neurons within the substantia nigra, specific regions of both the hypothalamus and hippocampus, the prefrontal cortex, and the cingulate gyrus.

In rodent models, including a VDR knockout model, it was demonstrated that vitamin D deficiency was associated with developmental abnormalities of the brain due to altered proliferation and differentiation[48]. Specific effects of vitamin D have been reported on the regulation of neurotrophin production such as stimulation of nerve growth factor (NGF) production and stimulation of glial cell line-derived neurotrophic factor (GDNF)[49]. Vitamin D has also been shown to inhibit nitric oxide production and decrease the production of various pro-inflammatory cytokines. In addition, a positive effect of $1.25(OH)_2D_3$ on the acetylcholine pathway has also been reported suggesting an effect on specific neurotransmitters[50]. The importance and clinical relevance of the VDR signaling within the central nervous system is still largely unclear. Early studies in animals have shown that vitamin D deficiency may increase the risk of brain dysfunction. In a recent study by Latimer et al., in aging rodents, a dose dependent improvement in cognitive function in response to vitamin D supplementation was seen. The suggested pathway was vitamin D mediated hippocampal gene expression that may improve the likelihood of successful brain ageing[51]. In a recent study, the offspring of dams who were fed a vitamin D deficient diet underwent a learning test. These mice from vitamin D deficient mothers exhibited

a learning disability at week 30 after birth. At week 70 after birth a significant loss in hippocampal volume was observed[52]. Recent observational studies link vitamin D status to specific neuro-psychiatric disorders in humans. In a study by Ganji et al, conducted in 7.970 persons aged 15 through 39, the risk of developing a depression was higher in individuals with a vitamin D deficiency[53]. In a study conducted in a group of older persons (aged 65-95 years) the risk of depression was associated with low serum $25OHD_3$ levels[54]. Studies also indicate a possible association of vitamin D deficiency with the development of Alzheimer's disease and Parkinson's disease[55]. In this thesis we aim to get more insight in the association between vitamin D status and cognitive functioning and studied vitamin D status in patients with symptoms of cognitive decline. This is described in more detail in chapter 3.3.

AIMS OF THIS THESIS

The objective of this thesis is to gain more insight into the pleiotropic functions of vitamin D in older adults, with an emphasis on mobility and neuromuscular function. In Section 2 the effects of ageing on the vitamin D endocrine system and calcium homeostasis are addressed. Ageing hampers the efficiency of these systems, leading to various health effects. Furthermore, the prevalence of vitamin D deficiency among older persons is studied and in a search for explanations for the persisting high prevalence of vitamin D deficiency among older persons the knowledge on vitamin D among older persons has been evaluated. In the last chapters of this section, trends regarding incidences of the most common osteoporotic fractures in the Netherlands were studied. In Section 3 the association between vitamin D status and functional performance is described. In the first chapter of this section the association between vitamin D and neuromuscular functioning is studied, and in the second chapter the effects of high dose vitamin D supplementation on neuromuscular function is investigated. In the third chapter the association between vitamin D and cognitive functioning is assessed. In Section 4 the associations between vitamin D status and vascular calcification are described. The possibility that specific arteries are affected differently by vitamin D deficiency is examined. In Section 5, the reflection on our

main findings, speculation on the implication of our results and indications for future research are presented.

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Section 2

Bone and calcium

Chapter 2.1

Ageing and vitamin D deficiency: effects on calcium homeostasis and considerations for vitamin D supplementation

Christian Oudshoorn, Tischa J. M. van der Cammen, Marion E. T. McMurdo, Johannes P. T. M. van Leeuwen and Edgar M. Colin

Br J Nutr. 2009; 9 Jun; 101(11): 1597-606

ABSTRACT

Vitamin D is a fat-soluble, seco-steroid hormone. In man, the vitamin D receptor is expressed in almost all tissues, enabling effects in multiple systems of the human body. These effects can be endocrine, paracrine and autocrine. The present review summarises the effects of ageing on the vitamin D endocrine system and on Ca homeostasis. Furthermore, consequences for vitamin D supplementation are discussed.

INTRODUCTION

Vitamin D is best known for its role in Ca homeostasis. Ageing affects both vitamin D metabolism and Ca homeostasis, with important consequences. In the present review, we outline new insights into the effects of ageing on both the vitamin D endocrine system and Ca homeostasis, which are relevant for clinicians who treat older people. Furthermore, considerations for vitamin D supplementation will be discussed.

VITAMIN D METABOLISM

Vitamin D is a fat-soluble, seco-steroid hormone. The term vitamin D refers to two precursors, i.e. cholecalciferol and ergocalciferol. Cholecalciferol is mostly formed in the skin after exposure to sunlight. In the skin, the precursor 7-dehydrocholesterol is transformed into cholecalciferol under the influence of short-wave UV light[1]. Another source of vitamin D is the diet. Ergocalciferol is generated in yeast and plants and cholecalciferol is produced in fish and mammals. In general, oral vitamin D intake, especially in Europe, is low and depends mostly on cutaneous production of vitamin D for our reserves[2]. The inert precursors are transported to the liver, where they are converted to 25-hydroxyvitamin D_3 ($25OHD_3$). In the kidney, $25OHD_3$ is hydroxylated by the enzyme $25OHD_3$-1α-hydroxylase (1α-OHase) to form 1,25 dihydroxyvitamin D_3 ($1,25(OH)_2D_3$), the most active vitamin D metabolite (Figure 1). 1α- OHase expression is not restricted to the kidney. Several cell types like macrophages, osteoblasts and neurons have also been shown to express 1α-OHase (Table 1)[3 – 5].

The primary function of the vitamin D endocrine system is maintaining Ca and phosphate homeostasis. Vitamin D stimulates both intestinal absorption and renal reabsorption of Ca and phosphate. Vitamin D deficiency results in decreased Ca and phosphate (re)absorption and subsequently lower serum levels of Ca and phosphate. This stimulates parathyroid hormone (PTH) secretion from the parathyroid glands[6]. PTH stimulates renal 1α-OHase expression and $1,25(OH)_2D_3$ formation. PTH also stimulates osteoclast formation (osteoclastogenesis). Osteoclasts stimulate bone resorption, releasing Ca and phosphate ions from the bone into the blood. A recent animal study has demonstrated that

osteoclastogenesis was increased in mice when serum $25OHD_3$ levels were < 80 nmol/l and this was positively associated with the receptor activator for NF-κB ligand/osteoprotegerin ratio. This increase in bone resorption was associated with the development of osteopenia and osteoporosis[7].

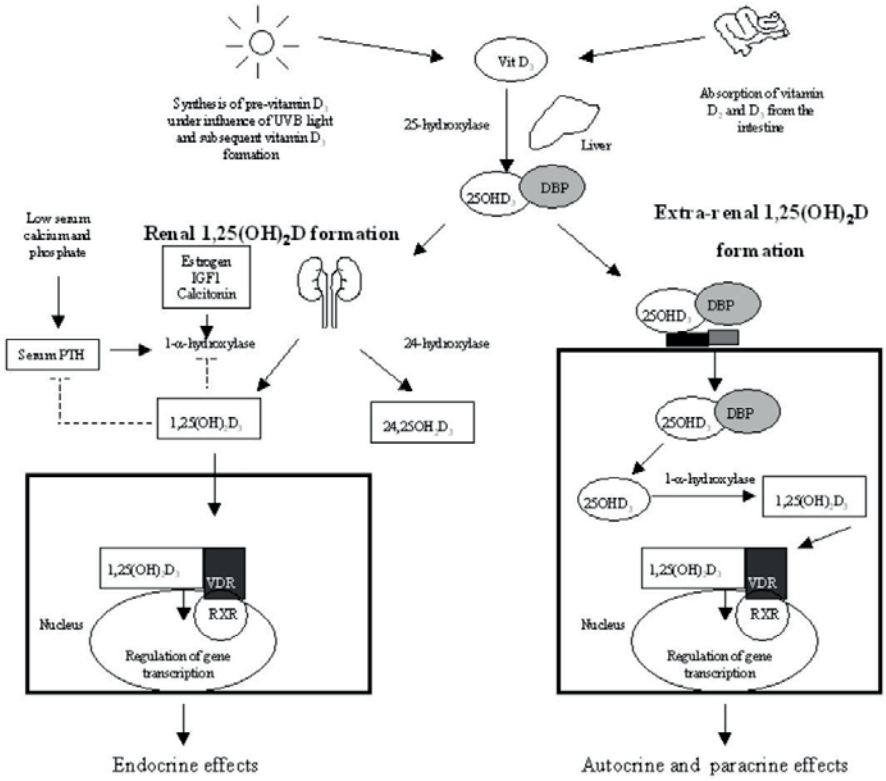

Figure 1. Different pathways for activation of vitamin D. Inert vitamin D precursors are either formed in the skin after exposure to UVB or derived from the diet. These precursors are hydroxylated in the liver to form $25OHD_3$. The $25OHD_3$ is bound to the vitamin D binding protein (DBP). The final 1-α- hydroxylation step which forms the most active vitamin D metabolite,$1,25(OH)_2D_3$ occurs either in the kidney (bulk) or in extra-renal cells expressing the 1-α-hydroxylase enzyme. The $1,25(OH)_2D_3$ formation in the kidney is tightly regulated via a feedback mechanism and the active vitamin D formed in the kidney exerts endocrine effects after binding to the vitamin D receptor (VDR). The VDR forms a heterodimer with the retinoid receptor (RXR) and regulates gene transcription. The active vitamin D formed extra-renally exerts paracrine and autocrine effects. IFG, insulin-like growth factor; FGF, fibroblast growth factor; PTH, parathyroid hormone; $24,25(OH)_2D_3$, 24,25-dihydroxyvitamin D_3

The optimal serum $25OHD_3$ level in human subjects to prevent stimulation of osteoclastogenesis is also believed to be about 80 nmol/l[7].

Other hormones that are known to stimulate renal 1α-OHase expression are insulin-like growth factor 1, calcitonin and oestrogen[8,9]. Increases in serum Ca, phosphate and $1,25(OH)_2D_3$ levels down-regulate renal 1α-OHase expression. Serum $1,25(OH)_2D_3$ levels are also regulated by the enzyme $25OHD_3$-24 hydroxylase (24OHase). Expression of 24OHase in the kidney is stimulated by $1,25(OH)_2D_3$ and this enzyme converts $1,25(OH)_2D_3$ into less active metabolites. These feedback mechanisms play an important role in the protection against hypercalcaemia and hyperphosphataemia[10].

Extra-renal 1α-OHase expression and activity is modulated differently from renal 1α-OHase and is less sensitive to feedback regulation by $1,25(OH)_2D_3$[11]. It is suggested that induction of extra-renal 1α-OHase involves regulatory pathways that differ from the renal, cyclic AMP-mediated pathway. For example, in osteoblasts, 1α-OHase expression and activity is not influenced by the levels of $1,25(OH)_2D_3$, PTH and Ca like renal 1α-OHase. IL1β, an activator of NF-κB, stimulates both 1α-OHase expression and activity in osteoblasts[4]. In macrophages, immune signals such as TNFα and interferon-γ modulate 1α-OHase expression, while in vascular smooth muscle cells extra-renal 1α-OHase expression and activity is stimulated by the hormones PTH and oestrogen (Table 1)[12,13]. Regulators of 1α-OHase expression and activity in most extra-renal tissues and the functions of the extra-renally formed $1,25(OH)_2D_3$ are still largely unknown. Some age-related effects on extra-renal 1α-OHase expression have been reported[14]. In an animal model, ageing resulted in decreased bone 1α-OHase expression[15]. Specific effects of ageing on the 1α-OHase expression and the activity in extra-renal tissues and health consequences of alterations in 1α-OHase expression and activity with ageing remain to be elucidated.

In recent years, several proteins have been discovered, which are important regulators of both Ca and phosphate homeostasis and the vitamin D endocrine system. These are fibroblast growth factor-23 (FGF-23) and klotho, a β-glucuronidase[16,17]. FGF-23 is involved in the regulation of renal phosphate excretion. FGF-23 inhibits expression of the renal sodium-phosphate transporter and thereby increases phosphate excretion[18]. In addition, FGF-23 decreases renal1α-OHase expression, stimulates 24OHase expression and decreases both PTH mRNA expression and PTH secretion from the parathyroid gland[18 – 20]. This leads to lower $1,25(OH)_2D_3$ levels and thus decreases vitamin D-related effects

on Ca and phosphate homeostasis. Klotho is involved in the regulation of renal Ca absorption and acts as a co-receptor or cofactor for other proteins such as FGF-23[21,22]. Klotho is capable of binding to various FGF receptors and enhances FGF-23 signalling. FGF-23 and klotho thus have important functions in regulating Ca and phosphate homeostasis and are important for skeletal health, both via effects on the vitamin D endocrine system and via direct, non-vitamin D-dependent effects.

Table 1. Extra-renal expression of the 1α-hydroxylase (1 α OHase) enzyme and effects of potential regulators relevant for ageing*

Cells and tissues expressing the 1α-OHase enzyme	Effects of potential regulators of 1α-OHase expression and/or activity
Placenta	$1,25(OH)_2D_3$: ↓ [107]
Keratinocytes	TGFβ1: ↑ [108] IFN- γ: ↑ [109], $1,25(OH)_2D_3$: - [110], TNFα : - [111], calcium: - [112], PTH: ↑ [112]
Pancreatic cells	-
Immune system (Monocytes/macrophages)	$1,25(OH)_2D_3$: ↑ [113], PTH: - [113], TNFα : ↑ [114], IFN-γ: ↑ [115]
Prostate cells	$1,25(OH)_2D_3$: ↓ [116], calcium: - [116] , PTH: - [116], EGF: ↑ [117], eostrogenic compounds: ↓ [118]
Osteoblasts	$1,25(OH)_2D_3$: - [4], PTH: - [4], calcium: - [4], IL 1β: ↑ [4], dihydrotestosterone: ↑ (in males) [14], eostrogenic compounds: (in females) [14]
Colon epithelium	$1,25(OH)_2D_3$: ↓ [119] , TGFβ1: ↑ (?) [120], dietary ca content: - [121]
Central nervous system	$1,25(OH)_2D_3$: ↑ [122]
Vascular endothelial cells and	TNFα : ↑ [123] , IFN-γ: [123]
Vascular smooth muscle cells	Eostrogenic compounds: ↑ [12] , PTH: [12]
Breast tissue	$1,25(OH)_2D_3$: ↓ [124] , $25OHD_3$: ↑ [125], eostrogenic compounds: ↑ [126]

* Based on ex vivo, in vitro and animal studies
$1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D_3; TGFβ1, transforming growth factor β1; IFN- γ, interferon- γ; PTH, parathyroid hormone; EGF, epidermal growth factor
↑ , Stimulating effect; ↓ , inhibiting effect; -, no efffect

EFFECTS OF AGEING ON VITAMIN D METABOLISM

Vitamin D deficiency is a worldwide problem[6]. Although in Europe and the United States there has been strong attention on vitamin D in recent years and vitamin D-fortified food products are widely available, vitamin D deficiency is still very prevalent among older people[23 – 25]. Mean serum $25OHD_3$ concentra-

tions in The Netherlands in independent community-dwelling older people are about 30 nmol/l and in institutionalised older people about 20–25 nmol/l[26,27]. Similar serum $25OHD_3$ levels among community-dwelling elderly have been reported in the United Kingdom and Germany[28,29]. Reports suggest that serum $25OHD_3$ levels in older people in the United States are higher than in Europe[26,30,31]. This is most likely due to higher oral intake of vitamin D in the United States where vitamin D fortification of food is more prevalent than that in Europe[24,32]. However, even in the United States >50% of the community-dwelling elderly are reported to have serum $25OHD_3$ levels < 75 nmol/l and about 30% of the elderly have levels < 50 nmol/l[30].

The high prevalence of vitamin D deficiency in older people may have several causes. Cholecalciferol synthesis in the skin after sun exposure is less effective in old age because of a decline in cutaneous levels of 7-dehydrocholesterol[33]. The level in a 70-year-old is only approximately 25% of the 7-dehydrocholesterol level in young persons[34]. This is worsened by the decreased exposure to sunlight with ageing due to immobility, lack of transport and social isolation[35,36]. Another factor contributing to the increased risk of vitamin D deficiency is an increase in body fat with ageing. The increase in fat mass leads to a larger distribution volume for the fat-soluble $25OHD_3$, which decreases the bioavailability of $25OHD_3$. Consequently, an inverse association has been demonstrated between BMI and both serum levels $25OHD_3$ and $1,25(OH)_2D_3$ and a positive association between BMI and PTH levels has been demonstrated[37,38].

An age-related decrease in $1,25(OH)_2D_3$ levels has also been suggested but reports are conflicting[39]. When vitamin D levels are low, compensatory hyperparathyroidism increases renal conversion of $25OHD_3$ to $1,25(OH)_2D_3$ and thereby maintains normal or even slightly elevated levels of this metabolite. As vitamin D deficiency worsens, $1,25(OH)_2D_3$ formation is impaired due to a lack of substrate[39]. Additionally, several age-related effects have been reported that could lead to lower $1,25(OH)_2D_3$ levels with ageing. First, renal function declines with age and this is accompanied with a decline in renal 1α-OHase activity and thus impaired conversion of $25OHD_3$ to $1,25(OH)_2D_3$[40]. Second, levels of insulin-like growth factor 1, calcitonin and oestrogen, which stimulate 1α-OHase expression and activity, decrease with ageing[41]. Furthermore, $1,25(OH)_2D_3$ metabolism may increase with ageing. In an animal model, an age-

dependent increase in renal 24OHase expression was reported. This occurred predominantly in female animals, suggesting an effect of ovarian hormones[42]. Ovariectomy in these animals was indeed associated with up-regulation of 24OHase expression. Interestingly, the induction of 24OHase by $1,25(OH)_2D_3$ may also be affected by ageing[43].

EFFECTS OF AGEING ON VITAMIN D ACTION

The active metabolite $1,25(OH)_2D_3$ exerts its function via the vitamin D receptor (VDR), a nuclear receptor. Upon binding of $1,25(OH)_2D_3$, the VDR forms a heterodimer with the retinoid receptor and binds to a vitamin D-responsive element in the promoter region of a target gene. This influences transcription of vitamin D-responsive genes[1]. In addition, the functions of the VDR are not limited to the binding to vitamin D-responsive element. The VDR has also been found to bind β-catenin, a key transcriptional factor in the Wnt signalling pathway[44,45]. This pathway has been implicated in a number of malignancies. By binding to β-catenin, the VDR blocks its transcriptional activity and so exerts antiproliferative properties. Besides genomic effects via the VDR, $1,25(OH)_2D_3$ also exerts non-genomic effects via a membrane-bound plasma receptor or second messengers such as cyclic AMP. These are rapid effects that do not depend on gene transcription.

Almost all tissues and cells in the body express the VDR, including those not directly involved in the regulation of Ca homeostasis, enabling a broad range of effects. Extra-renal $1,25(OH)_2D_3$ formation in various tissues implies that $1,25(OH)_2D_3$ is also capable of exerting paracrine and autocrine effects in addition to the well-known endocrine effects. Among the paracrine and autocrine effects are regulation of cell proliferation, differentiation and apoptosis[46].

Alterations in VDR expression leading to vitamin D resistance with ageing have received particular interest. With ageing, a decrease in VDR expression in bone, intestine and muscle tissue has been reported[47 – 49]. Various factors are known to influence VDR expression. Oestrogen, growth hormones and $1,25(OH)_2D_3$ are stimulators of VDR expression but serum levels decrease with ageing[50,51]. On the other hand, TNFα has been shown to down-regulate VDR expression, while serum TNFα levels increase with ageing[52,53].

In addition to a decrease in VDR numbers, binding of $1,25(OH)_2D_3$ to the VDR might also be decreased with ageing. A recent animal study, using competition VDR-binding assays with [^{3}H]-$1,25(OH)_2D_3$, has reported a decrease in $1,25(OH)_2D_3$ binding to the VDR with ageing in duodenal tissue[54]. Whether this also occurs in human subjects is not known.

PARATHYROID HORMONE AND AGEING

Like $25OHD_3$, PTH levels also exhibit seasonal variation with the highest PTH levels observed during the winter months[55]. A (secondary) rise in PTH levels is generally observed with ageing with a prevalence varying from 20 to 60%[56]. The most important causes of this secondary rise with ageing are vitamin D deficiency and resistance, renal insufficiency and low dietary intake of Ca[57]. PTH stimulates $1,25(OH)_2D_3$ formation and mobilises Ca from bone in order to maintain normal serum Ca levels[1]. Hyperparathyroidism not only negatively influences bone health but also is associated with sarcopaenia and falls as PTH stimulates muscle protein breakdown[58]. Furthermore, hyperparathyroidism has been related to cardiovascular events as PTH has been shown to promote vascular calcification[59]. Recently, elevated PTH levels have been shown to be an independent predictor of impaired long-term survival prognosis in older people[56]. High serum PTH levels (≥63 ng/l) were associated with significant increases in mortality (hazard ratio = 1.56, 95% CI: 1.29, 1.88) and a 2.3-year reduction of median life expectancy in a cohort of older patients[56].

CALCIUM HOMEOSTASIS AND AGEING

Ca homeostasis involves a coordinated control of Ca handling by the intestine, kidney and bone under the influence of primarily PTH and $1,25(OH)_2D_3$. Ageing, vitamin D deficiency and vitamin D resistance all affect these processes negatively. The two main mechanisms for Ca (re)absorption are a transcellular (active) and a paracellular (passive) route. The transcellular route involves entry of Ca into the cell at the apical side of the cell via Ca channels, diffusion of Ca through the cytosol bound to calbindins and active extrusion of Ca across the basolateral membrane via a Ca pump or a Na/Ca exchanger[57]. The epithelial

Ca channels are members of the transient receptor potential (TRP) super family and more precisely, the vanilloid subfamily (TRPV). The TRPV5 channel is the major isoform in the kidney, while the TRPV6 channel is highly expressed in the intestine. The paracellular route involves diffusion of Ca via tight junctions between epithelial cells.

Ageing and intestinal calcium absorption

An age-related decrease in intestinal Ca absorption has long been recognised[60]. In the search for age-related factors that explain this decrease in absorption, attention has focused on TRPV6. A TRPV6 mouse knockout model illustrated the importance of TRPV6 for intestinal Ca absorption. In TRPV6 knockout mice, intestinal Ca absorption was decreased by 60%[61]. Both in animal models and in human subjects, intestinal TRPV6 expression shows an age-dependent decline[48]. This is probably due to several effects as TRPV6 expression is regulated by $1,25(OH)_2D_3$, oestrogen, PTH and dietary Ca intake[57]. Recently, animal models have shed light on the importance of vitamin D metabolites for TRPV6 expression. Both in VDR and in 1α-OHase knockout mice, intestinal TRPV6 expression is strongly reduced, which impairs intestinal Ca absorption[62,63]. In addition, the ability of vitamin D metabolites to stimulate intestinal TRPV6 expression also seems to decrease with ageing[64].

The effects of ageing on TRPV6 expression differ among men and women. A recent study has reported that duodenal TRPV6 expression in both young and old men is strongly correlated with vitamin D status[48]. In women, however, TRPV6 expression decreased with ageing but no correlation was found with vitamin D status. In women, there was an age-dependent decline in VDR expression in the duodenal biopsies that was not found in men, which could account for the reduced vitamin D responsiveness and thus lower TRPV6 expression in women[48]. A possible explanation for decreased VDR expression could be decreased oestrogen levels with ageing. Oestrogen is important for vitamin D responsiveness as it stimulates both VDR and TRPV6 expressions[57]. Although the strongest decline in intestinal Ca absorption is seen after the menopause due to decreasing serum levels of oestrogen, another late age-related decrease in intestinal Ca absorption, in addition to the decline that occurs at the menopause, has also been reported in women after the age of 75[65]. This decrease

in intestinal Ca absorption of nearly 30% was independent of serum levels of $1,25(OH)_2D_3$ and $25OHD_3$ and of renal function. The cause of this late decline in Ca absorption, which is most likely due to increased vitamin D resistance, remains to be clarified.

In men, the importance of the sex hormone testosterone for Ca absorption is not well known and remains to be studied. A stimulating effect of testosterone on TRPV6 expression has been suggested[66].

Ageing and renal calcium reabsorption

Less is known about the TRPV5 Ca channel. Like TRPV6, an age-related decrease in TRPV5 expression has been reported[67]. Expression of TRPV5 is mainly regulated by $1,25(OH)_2D_3$, PTH and klotho[16,68]. Klotho is important for TRPV5 expression as it cleaves a carbohydrate residue from the Ca channel TRPV5, which increases TRPV5 expression and activity by trapping it in the plasma membrane[16]. Expression of klotho itself is positively regulated by $1,25(OH)_2D_3$ and oestrogen[69]. Several recent reports have demonstrated that klotho expression decreases with ageing[70,71]. In linking klotho expression to renal Ca absorption, it has been speculated that klotho deficiency may result in the down-regulation of TRPV5 expression and thus impairment of renal Ca reabsorption[72]. The importance of TRPV5 for renal Ca reabsorption has recently been demonstrated. TRPV5 knockout mice have severe hypercalciuria and decreased serum Ca levels[73]. Klotho knockout mice exhibit both decreased renal TRPV5 expression and decreased renal Ca reabsorption[74].

As serum Ca levels normally fluctuate between narrow margins, interplay between intestinal Ca absorption and renal reabsorption is required. A decrease in renal Ca reabsorptive capability is compensated for by an increase in intestinal absorption. A recent animal model has demonstrated that TRPV5 expression is an important determinant of TRPV6 expression. TRPV5 knockout mice have an increased intestinal TRPV6 expression and thus increased rate of intestinal Ca absorption[67]. In double TRPV5 and 1α-OHase knockout mice, the up-regulation of intestinal Ca transport was abolished suggesting that this is a vitamin D-dependent effect[75]. In patients with idiopathic hypercalciuria, a disease state characterised by decreased renal Ca absorption and high urine levels of Ca, a compensatory increase in $1,25(OH)_2D_3$ levels and intestinal Ca absorp-

tion is frequently observed[76]. The relevance of this interplay for maintaining Ca homeostasis in older people and effects of ageing remain to be studied.

Ageing and calbindins

Calbindins are cytosolic Ca-binding proteins. There are two major subclasses of calbindins: calbindin-D9k, which predominantly co-localises with TRPV6 in the small intestine, and calbindin-28k, which predominantly co-localises with TRPV5 in the kidney[57]. Calbindins act to facilitate the diffusion of Ca through the cell interior towards the basolateral membrane. By buffering Ca, calbindins protect cells against toxic effects during states of high Ca influx. Anti-apoptotic effects of calbindins have been reported in different tissues such as neurons, osteoblasts and pancreatic β cells[10]. Calbindin expression decreases with ageing, which could contribute to decreased Ca (re)absorption with ageing due to impaired transcellular diffusion[42]. This is also influenced by vitamin D deficiency as vitamin D stimulates calbindin expression in both the intestine and the kidney[10].

Other age-related effects on calcium absorption

PTH, besides stimulating intestinal Ca absorption via stimulation of renal 1α-OHase activity and thus 1,25$(OH)_2D_3$ formation and subsequently TRPV5 and TRPV6 expressions, also has direct effects on Ca absorption. The stimulation of duodenal Ca uptake by PTH has been demonstrated in an animal model[77]. In rat enterocytes, PTH enhances Ca influx through activation of the voltage-gated apical Ca channels and the cyclic AMP second messenger system. Interestingly, in aged duodenal cells, PTH is more efficient in stimulating Ca absorption when compared with duodenal cells of young rats[78]. This is most likely due to alterations in signal transduction via the PTH receptor that occur with ageing. It has been speculated that this increased efficiency is a compensatory mechanism in older people in states of impaired vitamin D status[57].

Another determinant of Ca absorption is the bioavailability of dietary Ca itself. Low-Ca diets increase the efficiency of intestinal Ca absorption. The activities of all known genes involved in the transcellular pathway are enhanced by low-Ca diets, probably via activation of the vitamin D endocrine system[57].

WHAT IS VITAMIN D DEFICIENCY?

Measurement of serum $25OHD_3$ level is the best clinical indicator to assess vitamin D status. Serum $25OHD_3$ levels represent the combined contribution of both cutaneous synthesis and oral intake of the various dietary sources of vitamin D[79]. Levels of $1,25(OH)_2D_3$ are less suitable to assess vitamin D status because even in a state of vitamin D deficiency $1,25(OH)_2D_3$ levels can be normal or slightly elevated.

With the ever-increasing insights into the effects of vitamin D, optimal vitamin D status is becoming more difficult to define. Criteria and cut-off values for vitamin D deficiency have mostly been linked to the effects of PTH levels on bone turnover. Serum $25OHD_3$ levels are inversely associated with PTH levels until an inflection point is reached. At this point, PTH levels begin to level off. Estimates for the serum $25OHD_3$ concentration at which the PTH concentration becomes constant vary from 25 to 122 nmol/l[80,81]. This wide variation in estimates is due to inter-individual variation in Ca (re)absorption and vitamin D responsiveness, as previously discussed. Old people generally require higher serum $25OHD_3$ levels, and thus vitamin D intake, to suppress PTH levels when compared with younger individuals. The capacity of the different vitamin D metabolites to raise serum $25OHD_3$ levels is unaltered with ageing[82].

When the effects on PTH levels and bone turnover are evaluated, serum $25OHD_3$ concentrations of > 50nmol/l are regarded by many as sufficient[83]. However, when other health benefits of vitamin D are taken into account, including its non-calcaemic effects, serum $25OHD_3$ concentrations of > 75nmol/l are advised[84]. In addition, the process of extra-renal $1,25(OH)_2D_3$ formation and autocrine and paracrine effects are most efficient when serum $25OHD_3$ levels are >75nmol/l[6].

In many trials that study the effect of vitamin D supplementation, Ca intake is not measured, which complicates the comparison of individual trial results. Dietary Ca content has been shown to modulate the $25OHD_3$/PTH association[81]. As Ca intake is lower, higher $25OHD_3$ serum levels are required to normalise PTH concentrations. In part, this may also explain discordant results between intervention trials with vitamin D, as Ca intake differs among countries[85,86].

CONSEQUENCES OF VITAMIN D DEFICIENCY AND RESISTANCE

Vitamin D deficiency and resistance have important consequences for older people (Figure 2). To illustrate its importance, vitamin D deficiency is associated with an increased risk for nursing home admission. The hazard ratio of nursing home admission after 6 years of follow-up for vitamin D-deficient individuals ($25OHD_3$ < 25 nmol/l) in a large cohort of older people was 3.48 (1.39–8.75) when compared with individuals with a high serum $25OHD_3$ level. The hazard ratio for vitamin D-insufficient individuals ($25OHD_3$ = 25–49 nmol/l) was 2.77 (1.17–6.55)[87]. The effects of vitamin D on bone, intestine and kidney, which are regarded as the classical target tissues, have been the subject of many studies for a long period of time. However, as the VDR is being found in increasingly more tissues, implications of vitamin D in many different disease states are be-

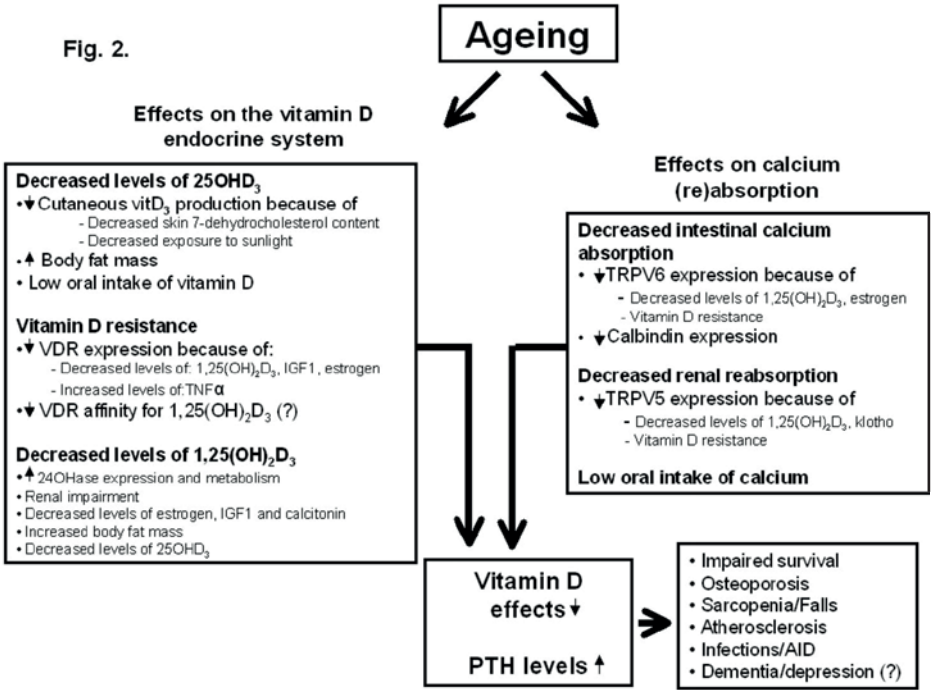

Figure 2. Consequences of ageing on both vitamin D endocrine system and calcium absorption. $25OHD_3$, 25-hydroxyvitamin D_3; VDR, vitamin D receptor; $1,25(OH)_2D_3$, 1,25-dihydroxyvitamin D; IGF, insulin-like growth factor; 24OHase, 24-hydroxylase; TRPV, transient receptor potential vanilloid; AID, auto-immune disorder

ing reported due to the effects of vitamin D outside these classical target tissues (Figure 2). Detailed effects of vitamin D have been reported on cardiovascular health, immune system, neurological diseases and cancer. The discussion of the effects of vitamin D in these disease states is beyond the scope of the present paper, but excellent reviews have recently been published[3,5,88,89].

Of note, recently adipose tissue has been shown to be a target tissue of vitamin D[90]. With ageing, there is an accumulation of fat in bone marrow at the expense of osteoblastogenesis, contributing to the development of senile osteoporosis. Vitamin D has been shown to block adipogenesis by inhibiting the expression of PPARγ2, a critical transcription factor for adipogenesis in bone marrow[90].

In general, the advancing knowledge of the effects of vitamin D in all these tissues further strengthens the call for adequate treatment of vitamin D deficiency[91].

TREATMENT OF VITAMIN D DEFICIENCY

Given the high prevalence of vitamin D deficiency in old age and the severe health consequences, a proactive approach from clinicians to case finding and adequate treatment of vitamin D deficiency is needed. Important considerations besides age are sex, BMI, skin colour, mobility, housing and dietary intake of both Ca and vitamin D[35,92,93]. Giving individualised treatment advice is complicated by the fact that the ideal vitamin D level has not yet been defined and the treatment effect on, for example, secondary hyperparathyroidism is also dependent on the dietary intake of Ca, which shows regional differences. In general, mobile, Caucasian community-dwelling elderly, who have a varied diet, need vitamin D supplementation of 10–20mg (400 IU–800 IU)/d to reach serum vitamin D levels of 50–75 nmol/l. Frail or institutionalised elderly on the other hand are suggested to need up to 50 mg (2000 IU)/d[84,94]. The effectiveness of this high-dose vitamin D supplementation in raising serum $25OHD_3$ levels adequately has been demonstrated in several clinical trials[95 – 97]. However, robust evidence on the optimal dose of vitamin D supplementation in specific high-risk groups is still lacking. In a recent report by the Dutch Health Council[98], 20mg (800 IU) daily is advised for high-risk groups, i.e., persons with osteoporosis, residents

of care homes, women aged 50+ and men aged 70+ with dark skin colour or housebound individuals.

Oral supplementation is the most effective intervention to treat vitamin D deficiency. Ergocalciferol is equally as effective as cholecalciferol in raising serum $25OHD_3$ levels[99]. Daily dosing is the most efficient interval to raise serum $25OHD_3$ concentrations when compared with weekly or monthly administration[26].

Although vitamin D supplementation therapy is generally regarded as safe, cases of iatrogenic and accidental overdose with cholecalciferol have been reported[100,101]. Most safety data concerning the use of high-dose cholecalciferol supplementation come from observations in relatively young individuals. Few studies have used high-dose cholecalciferol supplementation for longer periods in frail, older patients. Frail old people, particularly the institutionalised, often have poor daily fluid intake, use diuretics and have less thirst sensation than younger persons.

The need for high-dose supplementation therapy on the one hand, and the increased risk of dehydration on the other hand, may potentially increase the risk of accidental hypercalcaemia in these patients. Recently, concerns have risen regarding the possible negative health effects of vitamin D supplementation[88]. The recent discovery of FGF-23 and klotho has given more insight into possible negative health effects of vitamin D supplementation and hypervitaminosis D. In animal studies, both FGF-23 and klotho knockout mice have increased expression of the enzyme 1α-OHase. These mice have increased serum levels of $1,25(OH)_2D_3$, Ca and phosphate and have overall an identical phenotype. These knockout mice, despite their high levels of $1,25(OH)_2D_3$, develop osteoporosis, vascular and soft tissue calcifications, muscle wasting, pulmonary emphysema and have a shortened lifespan[102,103]. Klotho knockout mice have very high levels of FGF-23 (about 2000-fold higher), but have no sign of phosphaturia, illustrating the importance of klotho for FGF-23 signalling[18,21]. Normalisation of vitamin D activity in both klotho and FGF-23 knockout mice by either feeding them a vitamin D-deficient diet or knockout of the 1α-OHase enzyme increased survival and rescued most of the phenotype, illustrating that these effects are indeed vitamin D related[102,104]. Similar effects of hypervitaminosis D due to accidental overdose in human subjects have been reported[101,105]. Discontinu-

ation of vitamin D supplementation in human subjects with hypervitaminosis D due to excessive intake of vitamin D resulted in normalization of serum levels of $25OHD_3$, gradual recovery of bone density mineral and normalisation of the ratio of urinary Ca to creatinine[106]. So, when a patient is suffering from osteoporosis, clinicians should also consider, although rare, the possibility of vitamin D overdose.

CONCLUSIONS

Vitamin D is a pleiotropic hormone. Besides the effects on classical tissues like bone and intestine, vitamin D has an effect on many more tissues. Effects of vitamin D metabolites can occur via endocrine, paracrine or autocrine mechanisms.

Ageing increases the risk of vitamin D deficiency and is associated with vitamin D resistance and less efficient intestinal Ca absorption and renal reabsorption. Vitamin D supplementation doses needed to treat vitamin D deficiency and secondary hyperparathyroidism vary considerably between individuals. This makes it necessary for clinicians to give tailored advice to patients when treating hypovitaminosis D, taking into account these age-related effects and other characteristics that influence vitamin D status and Ca homeostasis. All clinicians who frequently treat older patients should take a proactive approach to screening at-risk individuals for vitamin D deficiency, as this condition is still very prevalent. When treating patients for vitamin D deficiency, Ca intake should be assessed. Possible unwanted effects of long-term vitamin D supplementation and the effects of hypervitaminosis D should be studied in forthcoming trials.

Acknowledgements: The manuscript was written by C. O. and E. M. C., T. J. v. d. C. and M. E. T. M. provided a critical review of the sections on the effects of ageing and treatment of vitamin D deficiency. J. P. v. L. provided a critical review of the section on the actions of vitamin D and the section on Ca homeostasis.
None of the authors had a personal or financial conflict of interest. This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

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Chapter 2.2

Better knowledge on vitamin D and calcium in older people is associated with a higher serum vitamin D level and a higher daily dietary calcium intake

Christian Oudshoorn, Klaas A Hartholt, Johannes P.T.M. van Leeuwen, Edgar M. Colin, Nathalie van der Velde and Tischa J.M. van der Cammen

Health Education Journal. 2011; 71 (4) 474-482

ABSTRACT

Objective: The objective of the present study was to examine knowledge on vitamin D and calcium in a cohort of older adults and to test the association between health knowledge, vitamin D status and dietary calcium intake.

Methods: The participants of this cross-sectional survey consisted of 426 individuals (≥65 years), living in residential homes. Participants were tested for their knowledge on vitamin D and calcium using a standardized questionnaire. Serum 25-hydroxyvitamin D_3 ($25(OH)D_3$) levels and dietary calcium intake were measured.

Results: The mean serum $25(OH)D_3$ level was 39.1 (±21.4) nmol/l and the mean daily dietary calcium intake was 826 (±242) mg/day. Of the participants, only 38 per cent indicated that they knew or had heard of vitamin D. Participants overestimated their daily calcium intake. Better knowledge on vitamin D and calcium was associated with both higher vitamin D levels ($P < 0.001$) and a higher daily dietary calcium intake ($P < 0.001$).

Conclusion: Given the poor knowledge on vitamin D and calcium and the observed associations, improving health knowledge could be a possible intervention to improve vitamin D status and calcium intake in older people. Further studies are needed to assess whether education will indeed lead to improvement of vitamin D levels and calcium intake in this age group.

INTRODUCTION

Osteoporosis is a skeletal disease characterized by low bone mass and micro-architectural deterioration, which results in an increased bone fragility and fracture risk[1]. The incidence of osteoporosis increases with age[2]. Important risk factors for osteoporosis are vitamin D deficiency and a low dietary calcium intake[3]. Especially among older people, vitamin D deficiency and low dietary calcium intake are very common[4]. A possible contributing cause may be a lack of knowledge on vitamin D and calcium in this age group. Health knowledge, or literacy, is an essential requirement to allow people to make health-conscious decisions. Older people are a high-risk group for low health literacy[5]. Data on knowledge among the general public, and older people in particular, about vitamin D and calcium is scarce. Studies that have been conducted are generally telephone or online surveys among relatively young individuals[6–8]. To the best of the authors' knowledge, no study has specifically evaluated knowledge on vitamin D and calcium among older people, or examined the association between knowledge, vitamin D status and calcium intake.

In order to gain more insight in the effect of health literacy in this age group, we tested the possible association between health knowledge, vitamin D status and dietary calcium intake in a cohort of older persons.

METHODS

Study participants

We performed a cross-sectional study among older people living in residential homes. Inclusion criteria were: no diagnosis of dementia (medical history) and aged 65 years and older. The study was approved by the Medical Ethics Committee of the Erasmus MC, University Medical Center Rotterdam (MEC-2007-160). Written informed consent was obtained from all participants.

Baseline characteristics

Participants underwent a clinical assessment including medical history, biography, current physical complaints and medication use, including supplements. Information on co-morbidities was obtained from the record of the residential home (physician) and this was cross-checked with information of the general practitioner.

Cognitive functioning was assessed using the Dutch version of the Mini-mental State Examination (MMSE), with a score ranging from 0 points (poor cognitive functioning) to 30 points (good cognitive functioning)[9]. Dietary food intake was assessed using a 24-hour dietary recall questionnaire. Calcium intake in milligrams per day was calculated using the Dutch National Food Composition Table[10]. In a randomly selected subgroup (~25 per cent) the 24-hour recall dietary assessment was repeated within several weeks after the initial visit. The correlation between the two measurements was 0.56, suggesting a moderate day-to-day variation, comparable with other reports[11]. Serum 25(OH)D_3 levels (in nmol/l) of all participants were measured using a radio-immuno-assay (DiaSorin, Stillwater, MN, USA).

Testing of knowledge on vitamin D and calcium

Participants were asked about their knowledge on vitamin D and calcium during a structured face-to-face interview by the principal investigator (CO). The questions asked are shown in Addendum 1. Participants who reported knowing about vitamin D (positive response on either question 1 (unprompted) or 2 (prompted)), were considered most knowledgeable on vitamin D (D+). Participants who responded negatively on both questions 1 and 2 were considered least knowledgeable on vitamin D (D–). Participants were considered most knowledgeable on calcium (Ca+) when they answered questions 2, 3 and 5 correctly (Addendum 1). Participants who did not answer these three questions correctly were considered least knowledgeable on calcium (Ca–).

Addendum 1. Questions asked

Knowledge on vitamin D

1. Name any vitamin you know
2. Do you know or have you heard of vitamin D (if not mentioned in question 1)?
3. Do you think your vitamin D status is sufficient?
4. What is the most important source of vitamin D?
5. Name dietary sources of vitamin D?
6. What is the most important effect of vitamin D?
7. Are you aware of the advice of the Dutch Health council regarding possible measures for the prevention of vitamin D deficiency?. If so, please name them.

Knowledge on calcium

1. Do you know or have you heard of calcium?
2. Name the most important dietary source of calcium?
3. What are the most important effects of calcium?
4. Do you think your dietary calcium intake is sufficient?
5. What is the daily recommended dietary calcium intake for individuals aged ≥ 70 years (in mg/day)?
6. Estimate your own daily dietary calcium intake (in mg/day).

Statistical analysis

Population characteristics were reported as mean ±SD. Baseline differences between the two groups (most or least knowledge) were tested using an independent t-test for normally distributed variables (age, gender and MMSE score) and the Mann–Whitney test for skewed variables (calcium intake and serum 25(OH)D_3 level). Secondly, the association between knowledge and vitamin D status and calcium intake was tested using linear regression analysis. To account for potential confounding, we computed a multi-variate model containing covariates that were considered biologically plausible (age, gender, education and MMSE score) or changed the point estimate by 10 per cent or more (none). Serum 25(OH)D_3 level and dietary calcium intake were natural log transformed given their skewed distributions. All statistical analyses were performed using SPSS software (version 16.1.1; SPSS Inc., Chicago, Illinois). A p-value of < 0.05 was considered statistically significant.

RESULTS

In total 460 individuals met the inclusion criteria and, of those, 426 (93 per cent) consented. The mean age of the participants was 81.0 years (±7.2), and 73 per cent were female. Further population characteristics are shown in Table 1.

Table 1. Characteristics of the study population

Characteristic	Participants (n = 426)	Range (min – max)
Age, yr	81.0 ± 7.2	65 – 103
Female, n (%)	315 (73 per cent)	-
Education, yr	8.7 ± 3.1	1 – 20
MMSE score (points)	26.5 ± 2.6	20 – 30
Serum 25(OH)D_3 (nmol/l)	39.1 ± 21.4	9 – 153
Dietary calcium intake (mg/day)	826 ± 242	226 - 1345
Use of vitamin D/calcium prescribed, n (per cent)	51 (12 per cent)	-
Use of vitamin D/calcium non-prescribed, n (per cent)	30 (7 per cent)	-

Mean ± standard deviation (SD)
MMSE: Mini-Mental State Examination

The mean (±SD) serum 25(OH)D_3 level was 39.1 (±21.4) nmol/l. Of the participants, 285 (67 per cent) had 25(OH)D_3 serum levels <50 nmol/l and 115 (27 per cent) had serum levels <25 nmol/l. The mean calcium intake was 826 (±242) mg/day. A total of 132 (31 per cent) participants had a calcium intake of <700 mg/day and 77 (18 per cent) had a calcium intake of >1200 mg/day.

Knowledge on vitamin D and calcium is shown in Table 2. Of the 426 participants, 106 reported knowing about vitamin D without prompting and 55 reported knowing about vitamin D after a prompt (total: n = 161/426; 38 per cent). Dietary products were considered as the most important source (n = 96; 60 per cent). All participants (n = 426; 100 per cent) had heard of calcium. A total number of 351 (82 per cent) participants knew that calcium is important for bone health and 329 (77 per cent) knew that dairy products are the main dietary source for calcium. The interrelation between estimated calcium intake and measured dietary calcium intake is shown in Table 3. In total 161 (38 per cent) participants were regarded most knowledgeable on vitamin D (D+). Likewise, 104 (24 per cent) participants were considered most knowledgeable on calcium (Ca+). The association between health knowledge and vitamin D status or calcium intake is shown in Figure 1.

Table 2. Knowledge on vitamin D and calcium

Knowledge on vitamin D		Knowledge on calcium	
Having heard/learnt about vitamin D (n = 426)		**Having heard/learnt about calcium (n = 426)**	
Unprompted	106 (25%)	Positive response	426 (100%)
Prompted	55 (13%)	**Dietary sources of calcium (n = 426)**	
Sources of vitamin D (n = 161; 38%)		Dairy products	329 (77%)
Diet	96 (60%)	Meat	23 (5%)
Sun exposure	49 (30%)	Fruit	16 (4%)
Don't know sources of vitamin D / Other	16 (10%)	Don't know sources of calcium / Other	58 (14%)
Dietary sources of vitamin D (n = 96)		**Most important effect of calcium (n = 426)**	
Dairy products	42 (44%)	Bone health	351 (82%)
Fish	22 (23%)	Don't know the effects / Other	75 (18%)
Don't know any dietary sources / Other	32 (33%)	**Estimation of own calcium intake (n = 426)**	
Most important effect of vitamin D (n = 161)		Thinks calcium intake is sufficient	218 (51%)
Bone health	54 (34%)	Thinks calcium intake not sufficient	153 (36%)
Immunity	76 (47%)	Don't know	55 (13%)
Mobility/muscle strength/falls	22 (14%)	**Estimation of the recommended daily dietary calcium**	
Don't know the effects / Other	9 (5%)	**intake in mg/day for individuals aged ≥ 70 years (n = 426)**	
Advice of the Dutch health council on the		< 700 mg	69 (16%)
prevention of vitamin D deficiency (n = 161)		700 mg – 1200 mg	95 (22%)
Heard of the advice and named correct measure	23 (14%)	> 1200 mg	107 (25%)
Didn't hear of advice or named wrong measure	138 (86%)	Don't know	155 (36%)

Table 3. Interrelation between estimated calcium intake by participants and measured dietary calcium intake by 24-h recall

Estimated daily dietary calcium intake: n	Measured daily dietary calcium intake n	
< 700 mg/day: 52	< 700 mg/day	42
	700 – 1200 mg/day	8
	> 1200 mg/day	2
700-1200 mg/day: 89	< 700 mg/day	23
	700 – 1200 mg/day	44
	> 1200 mg/day	22
> 1200 mg/day: 138	< 700 mg/day	49
	700 – 1200 mg/day	71
	> 1200 mg/day	18

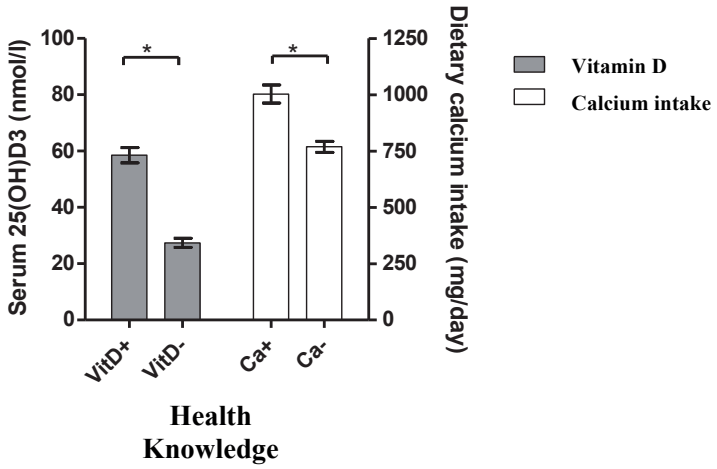

Figure 1. Baseline association of knowledge on vitamin D and calcium with vitamin D status and dietary calcium intake; * $p < 0.001$

Participants in the group D+ had a mean serum 25(OH)D_3 level of 58.5 (±17.7) nmol/l; in the group D– this was 27.4 (±13.5) nmol/l ($P < 0.001$). Among the participants that were considered D+, those who knew the advice of the Dutch Health Council on vitamin D had the highest serum 25(OH)D_3 levels (mean: 67.4 ±16.5 nmol/l). The dietary calcium intake in the Ca+ group (1003 (±208) mg/day) was significantly higher ($P < 0.001$) compared to the Ca– group (769 (±225) mg/day). Both the association between knowledge on vitamin D and serum 25(OH)D_3 levels and the association between knowledge on calcium and the daily dietary calcium intake remained significant after exclusion of people that used either vitamin D and/or calcium supplementation. For both knowledge on

vitamin D and knowledge on calcium, no association was observed with MMSE score or total years of education.

Table 4 shows the regression model of the association between health knowledge and vitamin D status and calcium intake. After adjustment for possible confounders, the associations between health knowledge and the health outcomes remained significant.

Table 4. Regression analysis of vitamin D status and calcium intake according to health knowledge

	Model 1			Model 2		
	B	SE	P-value	B	SE	P-value
Knowledge on vitamin D						
Serum 25(OH)D_3 level (nmol/l)*	0.837	0.049	< 0.001	0.816	0.049	< 0.001
Dietary calcium intake (mg/day)*	0.098	0.031	P = .002	0.075	0.031	P = 0.01
Knowledge on calcium						
Serum 25(OH)D_3 level (nmol/l)*	0.255	0.071	< 0.001	0.254	0.07	< 0.001
Dietary calcium intake (mg/day)*	0.288	0.032	< 0.001	0.286	0.032	< 0.001

Model 1: unadjusted
Model 2: adjusted for: age, gender, years of education, MMSE score
*Natural log-transformed

DISCUSSION

The associations between health knowledge and serum 25(OH)D_3 levels and dietary calcium were examined in a cohort of older persons. We demonstrated that better knowledge on vitamin D and calcium was associated with both higher 25(OH)D_3 levels and a higher daily dietary calcium intake. Only a third of the participants were familiar with vitamin D and knowledge of sources and effects of vitamin D was poor. The importance of calcium for bone health was generally well known, but most participants were falsely under the impression that their dietary calcium intake was sufficient.

Participants with the most knowledge on vitamin D (D+) had a serum 25(OH)D_3 level of 58.5 nmol/l, roughly twice as high as participants that had the least knowledge on vitamin D. The observed mean serum 25(OH)D_3 level of 39.1 nmol/l is in accordance with previous reports on the vitamin D status of older people, both from The Netherlands and other western countries[12,13]. In The Netherlands, serum 25(OH)D_3 levels of ≥50 nmol/l are currently recommended and in the United States levels of ≥75 nmol/l are advised[14,15].

The observed association was less obvious for individuals with either the most or the least knowledge on calcium. Although higher, the mean dietary calcium intake of 1003 mg/day in participants with the most knowledge on calcium (Ca+) was still below the recommended daily dietary calcium intake. The Dutch Health Council and the American Institute of Medicine currently recommend a daily calcium intake of 1200 mg/day for individuals ≥70 years[15,16]. While low, the mean dietary calcium intake of 826 mg/day in this cohort is still higher than reported calcium intakes in older people in other countries, such as England, France, the United States and Austria, illustrating the large regional and cultural variance in dietary calcium intake[11,17–19]. A large study from The Netherlands in non-institutionalized older people reported a daily dietary calcium intake of up to 1129 mg/day[20]. Possible explanations for the difference between the reported intakes in the study by Koek et al. and our study could be a difference in age and frailty between the studied participants[20].

The observed association between health knowledge and vitamin D status and calcium intake in our study indicate a promising intervention possibility. Improving health knowledge in old age groups using guidance by health professionals has been shown to be beneficial in raising awareness of health concepts[21]. In addition, a study by Engels et al. in a cohort of older persons demonstrated that informing participants about vitamin D increased the intention to start using vitamin D supplements[22]. The intention to start supplements was especially high when patients received this advice from their own physician. In contrast, a recent study in young women found no effect of increasing knowledge on vitamin D and calcium on dietary intake of vitamin D or calcium[23]. In addition, the interrelation between knowledge and behaviour can also be conflicting. For example, a previous study on sunlight and vitamin D revealed a conflict between knowledge and behaviour. Individuals who were best aware of the benefits of sunlight, and the importance of vitamin D for bone health, tended to avoid sunlight most by using sunscreen and parasols, and staying indoors[24]. This illustrates that ideally the effect of increasing health knowledge needs to be tested in a randomized controlled trial to ascertain a possible effect on vitamin D status and calcium intake. Multi-disciplinary teaching groups, taking into account a patient's specific background and experiences, could perhaps be an effective method[25].

Our study has several strengths. This is, to the best of our knowledge, the first study on knowledge about vitamin D and calcium that examined the participants' knowledge during a face-to-face interview with the use of standardized methods. This is also the first study that assessed the interrelation of health knowledge with vitamin D status and calcium intake. Another strength of the present study is that participants were unaware of the study and its goals before the initial visit. Consequently, participants were not able to prepare themselves for any of the questions. Furthermore, the participation rate of the individuals that were asked to participate in this study was high (93 per cent).

A limitation of the study is that because of the cross-sectional design, no causality can be concluded regarding the association between health knowledge and vitamin D status and/or calcium intake. For example, better knowledge in patients using vitamin D or calcium supplements could be due to the use itself rather than that participants with more health knowledge started using supplements. However, our finding remained significant after exclusion of participants that used vitamin D or calcium supplementation.

In conclusion, knowledge on vitamin D and calcium among this cohort of older persons was poor and this may have contributed to the high prevalence of vitamin D deficiency and low dietary calcium intake in this age group. In view of the severe consequences of vitamin D deficiency in old age, we feel that it is important to better inform older persons about the role and necessity of vitamin D and calcium, and to improve knowledge on current guidelines and possible preventative measures. Further studies are, however, needed to assess whether education will indeed lead to improvement of vitamin D levels and calcium intake in this age group.

Acknowledgments: Klaas Hartholt is a research fellow at the Erasmus MC, appointed on a research grant from 'The Netherlands Organization for Health Research and Development' (ZonMw), project number 170.885.607. The funders did not have any influence in study design, in the collection, analysis, and interpretation of data, in the writing of the report or in the decision to submit the paper for publication. The authors' specific responsibilities within this study are as follows. CO: study design, data acquisition and analysis, drafted paper; KH: drafted paper, data analysis, critical revision; JvL: interpretation of the data,

critical revision, supervision; EC: interpretation and statistical analysis, critical revision; NvdV: data analysis and interpretation, critical revision; TvdC: study design, critical revision, supervision.

Funding: This research received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

Conflict of interest statement: The author(s) declared no conflicts of interest with respect to the authorship and/or publication of this article.

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Chapter 2.3

The epidemic of hip fractures, are we on the right track?

Klaas A. Hartholt, Christian Oudshoorn, Stephanie M. Zielinski, Paul T.P.W. Burgers, Martien J.M. Panneman, Ed. F. van Beeck, Peter Patka and Tischa J.M. van der Cammen

PLoS One. 2011; 6(7):e22227

ABSTRACT

Background: Hip fractures are a public health problem, leading to hospitalization, long-term rehabilitation, reduced quality of life, large healthcare expenses, and a high 1-year mortality. Especially older adults are at greater risk of fractures than the general population, due to the combination of an increased fall risk and osteoporosis. The aim of this study was to determine time trends in numbers and incidence rates of hip fracture-related hospitalizations and admission duration in the older Dutch population.

Methods and Findings: Secular trend analysis of all hospitalizations in the older Dutch population (≥65 years) from 1981 throughout 2008, using the National Hospital Discharge Registry. Numbers, age-specific and age-adjusted incidence rates (per 10,000 persons) of hospital admissions and hospital days due to a hip fracture were used as outcome measures in each year of the study. Between 1981 and 2008, the absolute number of hip fractures doubled in the older Dutch population. Incidence rates of hip fracture-related hospital admissions increased with age, and were higher in women than in men. The age-adjusted incidence rate increased from 52.0 to 67.6 per 10,000 older persons. However, since 1994 the incidence rate decreased (percentage annual change −0.5%, 95% CI: −0.7; −0.3), compared with the period 1981–1993 (percentage annual change 2.3%, 95% CI: 2.0; 2.7). The total number of hospital days was reduced by a fifth, due to a reduced admission duration in all age groups. A possible limitation was that data were obtained from a linked administrative database, which did not include information on medication use or co-morbidities.

Conclusions: A trend break in the incidence rates of hip fracture-related hospitalizations was observed in the Netherlands around 1994, possibly as a first result of efforts to prevent falls and fractures. However, the true cause of the observation is unknown.

INTRODUCTION

Fall incidents and fall-related injuries among older people are a major public health problem in ageing societies worldwide[1-3]. Of people aged ≥65 years approximately one third fall each year[4-7]. Especially older individuals are at an increased risk of sustaining fractures after a low energetic trauma, e.g. a fall incident, due to underlying medical conditions, especially osteoporosis[8].

Osteoporosis, a highly prevalent condition in the older population, is characterized by low bone mass and micro-architectural deterioration of bone tissue. Osteoporosis results in an increased bone fragility and increased susceptibility to fractures[8]. Typical sites of osteoporotic fractures include those of the hip, wrist, vertebrae, and upper arm[9]. Approximately 85% of all hip fractures occur in individuals aged ≥65 years[10]. Hip fractures are, more than any other type of fracture, associated with a loss of independence[11], morbidity[12], and mortality[13].

Besides the health impact on the individual patient, the socioeconomic impact of osteoporosis and of hip fractures in particular is substantial[14]. Hip fractures are currently leading to nearly half (46%) of all injury related healthcare costs in older adults in the Netherlands[15,16]. In a global perspective, the annual estimated worldwide direct and indirect costs of hip fractures amounted to $34.8 billion in 1990, and are expected to rise to an estimated $131 billion by 2050[17]. With the expected continuing ageing of populations worldwide[18], it might be expected that the number of hip fractures will increase accordingly, making it necessary to prepare our healthcare systems for this burden.

In order to optimize healthcare use and healthcare planning in an ageing society, accurate numbers in hip fracture incidence are mandatory. The aim of this study was to provide secular trends of age- and gender specific numbers, incidence rates and length of hospital stay (LOS) of hip fractures in the older Dutch population.

MATERIALS AND METHODS

For this study all data of hospital admissions due to a hip fracture in persons aged ≥65 years were collected from 1981 throughout 2008 in the Netherlands. The data were retrieved from Statistics Netherlands (CBS, The Hague, The Netherlands),

which combines information of the National Medical Registration (LMR)[19] and the National Hospital Discharge Registry. Data regarding hospital admissions, admission diagnosis, LOS in days, age, and gender are stored in this database. The LMR database has a high nationwide coverage and nearly all admissions are stored in this database (less than five percent missing). Hospital admissions data and population numbers were verified with the national Birth-Registry[19]. The Birth-Registry is used to identify individual patients in the National Medical Registry. Data were corrected for missing values by the Statistics Netherlands, and extrapolated to full national coverage[20]. A uniform classification and coding system is used by the LMR for all hospitals and did not change during the study period. Official coding clerks register the diagnosis and injury mechanism of all hospital admissions, based on data obtained from medical records. Throughout the study period, a hip fracture was defined by using the International Classification for Diseases, 9th revision of the World Health Organization, code 820. Older persons were defined as persons aged 65 years and older. Demographic numbers were retrieved from the Statistics Netherlands. In this study the mid-year population was used. The medical ethical review board of the Erasmus MC, University Medical Center, Rotterdam, approved the study (MEC-2010-402) and provided a waiver for 'informed consent', because the data were retrieved from a large public accessible database, containing anonymous data on admissions, which cannot be traced to individuals.

Numbers of hospitalizations due to hip fractures were specified for age and gender. The age-specific incidence rates were calculated in 5-year age groups using the number of hip fractures in that specific age group, divided by the population size within that specific age-group for male and female patients, and was expressed per 10,000 persons in that age-group. Age-adjusted incidence rates allowed us to compare the incidence rate for a standardized population during the study period, and were performed by 'Direct Standardization' to correct for demographic changes throughout the study period. Growth in the numbers of hospital admissions and LOS were calculated in percentages compared to the index year 1981. Data were analyzed using a Poisson regression analysis for annual growth in overall hospital admissions for older persons, corrected for population size and age composition. In order to model the trend in hospital admissions, a linear regression model with Poisson error and log link was built with log (mid year population size of each year of the study) as offset factor. To assess if the

annual growth changed during the study period for both genders, the Joinpoint Regression Program, Version 3.4.3. (Statistical Research and Applications Branch, National Cancer Institute, USA) was used. This program showed the necessity for assuming a spline instead of a simple linear model, for men and women separately, and determines where to place the knot. The spline function accommodated two piecewise linear fits, connected with one another at the knot. Comparison of these two periods enabled us to detect and quantify changes in the secular trend in admission rates such as stagnation or an increase in admission rates. The best knot was found to be January 1, 1994. The parameter for calendar year, corrected for gender and age-group was transformed into Percentage Annual Change (PAC). The analysis including splines yielded estimates of annual changes in admission rates within each period (1981–1993 and 1994–2008). All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software (version 16.1.1). A p-value <0.05 was considered statistically significant.

RESULTS

During the study period from 1981 throughout 2008, 355,320 patients aged ≥65 years were admitted due to a hip fracture in the Netherlands. The annual number of hip fracture-related hospitalizations doubled in both men and women, from 7,614 cases in 1981 to 16,049 cases in 2008 (Table 1). The male:female ratio remained 1:3 throughout the study period. The crude incidence rate increased, from 46.4 per 10,000 older adults in 1981 to 66.5 per 10,000 in 2008 (an increase of 43.3% compared to 1981), and peaked in 1995 (70.4 per 10,000 older adults).

Table 1. Population characteristics of persons aged ≥65 years, number, incidence and mean admission duration of hip fracture-related hospitalizations in persons aged ≥65 years (The Netherlands, 1981-2008)

Characteristic	1981	1986	1991	1996	2001	2006	2008
Population ≥65 yr (x 1,000)	1,642	1,769	1,934	2,061	2,175	2,330	2,415
Female (%)	59.0%	61.2%	60.2%	59.8%	58.9%	57.6%	57.0%
Admissions overall (n)	7,614	9,958	12,565	14,508	14,810	15,249	16,049
- men (n)	1,857	2,281	2,879	3,326	3,385	3,845	4,105
- women (n)	5,757	7,677	9,686	11,182	11,425	11,404	11,944
Incidence rate†	46.4	56.3	65.0	70.4	68.1	65.4	66.5
Admission duration (days)	37.0	32.1	30.0	23.8	23.1	15.4	14.0

† Crude incidence rate, expressed per 10,000 older adults

For older men the crude incidence rate increased from 27.6 to 39.5 (an increase of 43.3%) and for older women from 59.5 to 86.8 (an increase of 46.0%) from 1981 to 2008 respectively.

Gender and age-specific incidence rates of hip fracture-related hospital admissions are shown in Table 2. For men and women aged 65–74 years the age-specific incidence rates of hip fractures did not change significantly when comparing 2008 to 1981. However, a strong increase (>50%) in the incidence rate of hospital admissions due to hip fracture was seen in men aged ≥80 years since 1981, up to an increase of 127% in men aged ≥95 years (from 156.3 per 10,000 in 1981 to 354.7 per 10,000 in 2008). Age-specific incidence rates for women aged ≥75 years showed growth of one sixth to a quart.

The overall age-adjusted incidence rate of hip fractures increased (Figure 1) from 52.0 per 10,000 older adults in 1981 to 62.7 in 2008 (an increase of 20.6%). Throughout the study period, the age-adjusted incidence rate for women (68.6 per 10,000 older women in 1981 and 79.9 in 2008) remained twice as high compared to men (27.9 per 10,000 older men in 1981 and 37.8 in 2008).

The PAC, change per year, of the age-adjusted incidence rate was 1.13% (CI 95%: 0.80; 1.45) for men versus 0.52% (CI 95%: 0.24; 0.81) for women over the whole study period. A joint-point regression analysis showed that the change in age-adjusted incidence rates was not constant over time and could be divided into two phases: first, the incidence of hospital admissions due to a hip fracture in older patients increased between 1981 and 1993, and second, decreased between 1994 and 2008 (Figure 1). The annual growth in men was 2.46% (CI 95%: 1.98; 2.94) and in women 2.16% (CI 95%: 1.89; 2.43) in the period 1981–1993. The PAC decreased in the period 1994–2008 to a negative annual growth of –0.34% (CI 95%: –0.86; 0.19) in men and –0.64% (CI 95%: –0.83; –0.46) in women.

Also the mean LOS decreased throughout the study period in both men and women, from 37.0 days in 1981 to 14.0 days in 2008 (Figure 2).

The admission duration decreased over 60% in male and female patients of 65–79 years. Reduction in LOS was smaller in the older patient groups. In patients ≥80 years the LOS per admission was reduced by a third. In general, the LOS was age-related: the higher the age, the longer the admission duration (Figure 2). Although the total number of hip fracture-related hospital admissions increased, the total number of hospital-bed-days decreased due to a reduced

Table 2. Incidence of hip fracture-related hospital admissions per 10,000 persons in males and females, the Netherlands (1981-2008)

	65-69 year		70-74 year		75-79 year		80-84 year		85-89 year		90-94 year		≥95 year	
Year	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women	Men	Women
1981	10.2	16.6	16.6	29.5	31.9	58.8	57.7	113.1	89.7	209.8	156.2	272.4	156.3	319.4
1986	11	19.6	20.3	34.6	34.8	66.9	65.1	128.6	107.3	229.7	190	303.5	233.7	349.2
1991	12.2	21.7	20.7	40	41.9	78.9	77.9	134	141.1	235.5	220.4	369.9	296.4	379.3
1996	12.2	21.8	24.4	45.5	45.6	82	86.3	147.2	148.1	240	211	363.5	269.0	410.9
2001	9.1	18	18.4	38.3	45.1	81.5	80.9	144.6	148.3	241.4	237.3	338.1	345.1	369.3
2006	9.8	15.8	16.9	32.5	38.2	72.1	85.3	138.2	159.6	229.7	265.5	326.1	372.3	385.1
2008	9.1	16.8	18.1	32.2	38.9	70.1	81.1	139.3	161.6	237.5	275.7	325.5	354.7	388.2
Change*	-11%	1%	9%	9%	22%	19%	41%	23%	80%	13%	77%	19%	127%	22%
(95% CI)	(-24; 5)	(-10; 14)	(-5; 26)	(-1; 20)	(8; 37)	(11; 28)	(25; 58)	(16; 31)	(58; 106)	(6; 21)	(47; 112)	(8; 32)	(55; 233)	(0; 48)

*change is 2008 compared to 1981; 95% CI:, 95% Confidence Interval

LOS per admission. The total numbers of hospital-bed-days are shown in Figure 3 and decreased from 281,396 days in 1981 to 224,002 days in 2008 (a decrease of 20%).

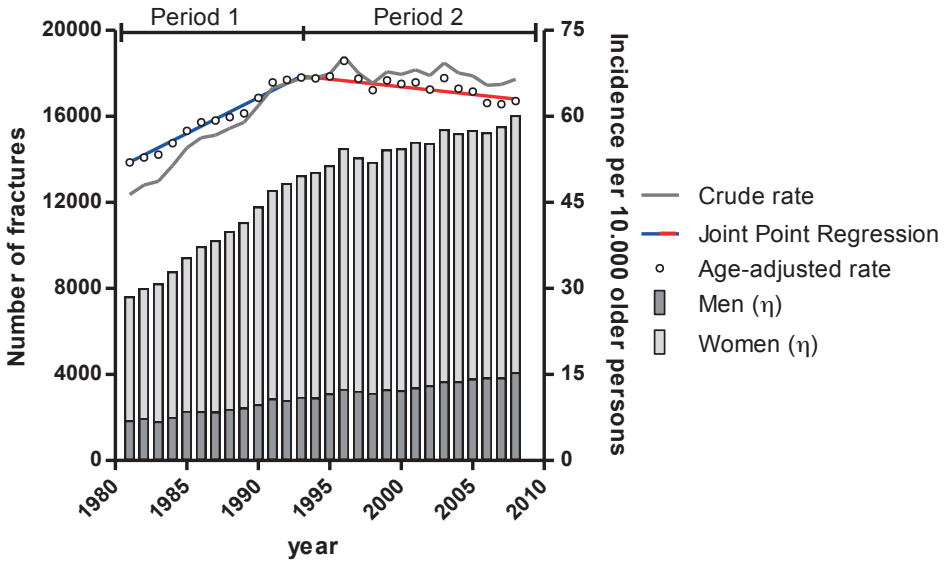

Figure 1. Absolute numbers, crude and age-specific incidence rates of hip fracture-related hoppitalizations in the Dutch population population ≥65 years (1981-2008). Period 1 (dashed blue line): 1981-1993, percentual annual change 2.30%, (95% CI: 2.00; 2.59). Period 2 (dashed red line): 1994-2008, percentual annual change -0.50% (95% CI: -0.70; -0.30)

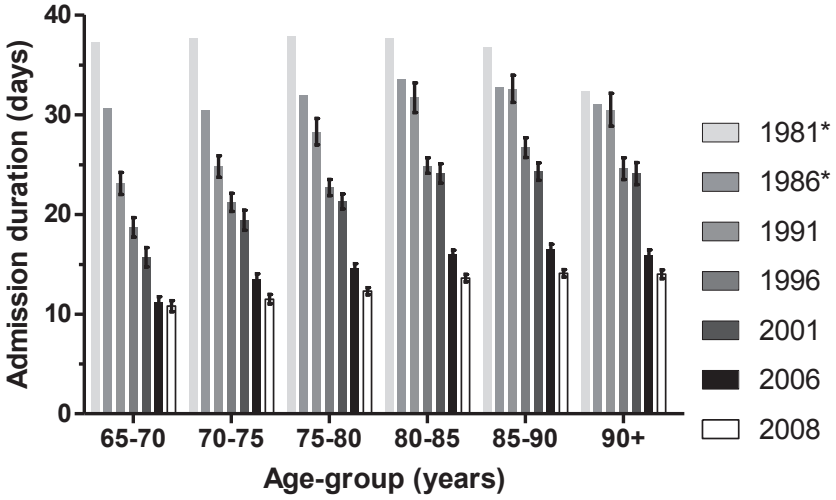

Figure 2. Mean hospital admission duration in persons aged ≥65 years due to a hip fracture in the Netherlands between 1981-2008.* No SD data was available before 1991

Figure 3. Total number of hip fracture-related hospital-bed-days in persons of ≥65 years in the Netherlands between 1981-2008

For all men aged ≥65 years, the total number of hospital days decreased with 8% (from 62,980 days in 1981 to 58,146 days in 2008). In women aged 65–79 years, a reduction of 54% in hospital days was seen (from 94,903 days in 1981 to 43,474 days in 2008). In women aged ≥80 years the number of hospital days increased until 1991 to 194,264 days and from there on started to decrease, with the total number of hospital days in 2008 (122,382 days) just below (−1%) the total number of hospital days in 1981 (123,513 days).

DISCUSSION

In order to determine trends in hip fractures in the older Dutch population, all hip fracture-related hospitalizations were analyzed from 1981 throughout 2008. The age-adjusted incidence rates of hip fractures increased until the end of 1993 in the population ≥65 years. After that year, a trend break was observed and the incidence rates started to decrease. Although an encouraging decrease in the age-adjusted incidence rates was observed, the absolute number of hip fractures continued to increase due to a rising number of older persons in the population.

Comparable trends of decreasing incidence rates for hip fracture-related hospitalizations since the mid-nineties have been reported in several countries around the globe, such as the United States[21], Canada[22], and Finland[23]. However, not all findings across western countries are consistent. A recent study from Germany failed to demonstrate a decline in hip fracture incidence rates[24]. Since most studies on hip fracture incidence from multiple countries point in the same direction, there might be a causal explanation for this observation. However, there is no simple answer to this question, because risk factors for hip fractures are multifactorial, as mentioned by Leslie et al.[22] Important developments over the last two decades include: the increasing awareness of falls[25,26], the implementation of guidelines for the diagnosis and treatment of osteoporosis[27,28], increasing availability and use of bisphosphonates[29], and an improvement of calcium intake and vitamin D status, although the latter is argued by some[22]. Other nationwide changes, such as the prevention and improved treatment of cardiovascular diseases in the general population may also have contributed to the observed trend break. A large Finnish twin-study recently demonstrated that cardiovascular diseases are associated with the development of hip fractures[30]. However, the exact mechanism behind this association is not clear yet[30]. Another possibility might be that general health[31] and bone quality[32] have improved since smoking has been discouraged. The proportion of smokers is decreasing rapidly in the Netherlands[31,33] as well as in other countries[34,35]. Furthermore, the Statistics Netherlands (CBS) reported that the mean body weight has increased in the Dutch population[33]. An increased Body Mass Index is associated with a lower fracture risk[36].

A remarkable difference was observed between the younger and older age-groups. Whereas incidence rates decreased in persons <80 years, the incidence rate stabilized in females aged ≥80 years, and continued to increase in males ≥80 years. This finding is worrisome because the population of 80 years and over is the fastest growing segment in the ageing population[37] and because mortality and morbidity associated with hip fractures are greater for the oldest old, and are higher in men than in women in the first year after sustaining a hip fracture[38,39]. A possible explanation for this observation might be that life expectancy increased more rapidly in men compared to women over the past decades, resulting in a smaller gap in life expectancy between men and

women[40]. Consequently, men have become more vulnerable for age-related (co)morbidities, such as osteoporosis and hip fractures, which were previously frequently seen in older women. This assumption is supported by a previous report on a more rapid increase in fall-related injuries, hospitalizations, and mortality in older men than in older women in the Netherlands over the past decades[1,41]. Another possible explanation might be that osteoporosis in men is frequently underdiagnosed and undertreated[42].

The number of hospital-bed-days per admission is considered to be one of the most important determinants of total costs per hip fracture in an individual patient[43]. Therefore, a reduced LOS is necessary in order to reduce hospital care demands and to limit related healthcare costs. During the study period the LOS decreased by two-thirds. Several factors might have contributed to this impressive reduction: the rapid improvement of surgical and anesthetical care over the last decades, resulting in less invasive surgical procedures; the introduction of new hip prostheses, and implants; protocols for early timed surgery after a hip fracture; better pain management and better post-operative care with early mobilization; early discharge to designated rehabilitation places and skilled nursing homes; and the implementation of hip fracture treatment guidelines[44-46]. In addition, during the final years of the study period a change in the financing structure of Dutch hospitals, which was introduced in 2004, may have led to a further decline in LOS.

A strength of the present study is the availability of population-based in-hospital data, covering a period of 28 years. The Dutch healthcare system is characterized by full health insurance coverage and full accessibility for the whole population during the study period. Since 1981 absolute numbers of hip fracture related hospital admissions and hospital-bed-days in all hospitals in the Netherlands have been recorded with nearly complete national coverage in a highly accurate electronic database. Throughout the study period, the coding system of the National Medical Registry did not change and no major policy changes were introduced in the Netherlands which might have affected the increase in admission rates. However, this study has some limitations. A possible limitation is that these data describe the situation in one country, which may not directly translate to other western countries, because of differences in healthcare system characteristics and demographics. Nevertheless, since

hip fracture trends[21,22,47] in other western populations are comparable with the trends in the Netherlands, there is no reason to assume that hip fractures trends will be substantially different in other countries. This study is based on a linked administrative database, which does not contain clinical data regarding underlying diagnosis, co-morbidity, injury severity, lifestyle, or medication use of the patients. This limits the interpretation of the causal mechanisms behind the observed trends. Furthermore, readmissions in one calendar year were not excluded and could potentially lead to some 'double registration'. However, it is unlikely that readmissions influenced our results, since readmissions for injuries constitute at the most 2.6% (at the maximum) in the Netherlands, as was found in a study by Polinder et al[48].

In summary, the increase in hip fracture incidence rates slowed down between 1981 and 1993, and the incidence rates started to decrease over the last 14 years. However, incidence rates nowadays remain higher than in 1981, suggesting that there is still room for improvement. Furthermore, the continuing increasing incidence rates in the oldest men is a worrying trend that deserves specific attention, since the group of persons aged 80 years and older are the fastest growing segment of aging societies. With the expected ageing of societies worldwide, continued attention is needed in order to cope with the demand of hip fracture related care in the near future.

Acknowledgments: Klaas Hartholt is a research fellow at the Erasmus MC, appointed on a research grant from "The Netherlands Organization for Health Research and Development" (ZonMw), project number 170.885.607.

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Chapter 2.4

Emergency department visits due to vertebral fractures in the Netherlands, 1986-2008: Steep increase in the oldest old, strong association with falls

Christian Oudshoorn, Klaas A. Hartholt, M. Carola Zillikens, Martien J.M. Panneman, N. van der Velde, Edgar M. Colin, Peter Patka and Tischa J.M. van der Cammen

Injury. 2012; Apr;43(4):458-61

ABSTRACT

Background: Vertebral fractures are a common consequence of osteoporosis in older persons. With the ageing of the population, numbers are expected to rise.

Objective: To determine trends in health care demand due to vertebral fracture related emergency department (ED) visits and hospitalizations in the older Dutch population.

Design and setting: Secular trend analysis of vertebral fracture related ED visits between 1986 and 2008, using the Dutch Injury Surveillance System. All ED visits with a primary diagnosis of a vertebral fracture in persons aged ≥65 years were extracted from this database. Main outcome measure: Numbers, age-specific and age-adjusted incidence rates (per 100,000 population) of ED visits and hospitalization rates due to vertebral fractures in the older Dutch population were calculated for each year of the study.

Results: The total number of ED visits due to a vertebral fracture increased from 913 in 1986 to 2,502 in 2008 (174% increase). The majority of fractures were caused by a low-energetic fall incident (83%). The overall age-adjusted incidence rate increased from 51.6 per 100,000 population in 1986 to 103.6 in 2008. Incidence rates increased with age and were higher in females than in males. The hospitalization rate remained stable at about 50-55%, in both females and males.

Conclusion: Vertebral fracture related ED visits and hospitalizations are increasing rapidly in the older Dutch population, especially in the oldest-old. Most vertebral fractures were associated with falls. These findings indicate that a pro-active approach in the diagnosis and treatment of osteoporosis and in the prevention of falls in both men and women is warranted.

INTRODUCTION

Osteoporosis is a growing public health concern in developed countries worldwide[1]. It is a skeletal disease, characterized by low bone mass and micro-architectural deterioration, which results in an increased bone fragility and fracture risk[2]. Vertebral fractures are one of the most common osteoporotic fractures. Approximately 90% of vertebral fractures are associated with osteoporosis[3]. Vertebral fractures lead to functional impairment, impaired quality of life and increased mortality[4-7]. A previous vertebral fracture is associated with an increased risk of further vertebral fractures and hip fractures[8].

It was estimated that in the year 2000, nine million osteoporotic fractures occurred worldwide, and of these approximately 1.4 million (15%) were clinical vertebral fractures[9]. The majority of vertebral fractures however are morphometric, i.e. clinically silent. The prevalence of radiographically identified vertebral deformities has been estimated to be 5% between the age of 50-54 years, and rises to 50% at age 80-84 years[10].

The number of patients with a vertebral fracture is expected to increase because of the increasing life expectancy and the increasing number of osteoporotic individuals in the population[11-13]. However, there are few data on time trends of healthcare demand due to clinical vertebral fractures. The aim of this study was to analyze time-trends in clinical vertebral fractures by analyzing trends in emergency department (ED) visits and hospitalization rates after ED visit.

METHODS

Data on ED visits due to a vertebral fracture in the Dutch population aged 65 years and over was extracted from the Dutch Injury Surveillance System (LIS). The LIS database is a continuous monitoring system in which injury diagnoses and injury mechanisms are registered by using the International Classification of Diseases of the World Health Organization (ICD 10th revision)[14]. LIS is based on 13 geographically distributed EDs in the Netherlands, resulting in a representative 12% sample of injury-related ED visits. Numbers were extrapolated to national estimates. An extrapolation factor was calculated by the Consumer and

Safety Institute (Amsterdam, the Netherlands) based on the adherent population of the participating hospitals and Dutch population numbers in each year of the study. The database makes it possible to measure and describe healthcare use during a specific period. The full-model description has been published by the Consumer and Safety Institute, Amsterdam and has been used previously[15-17].

The model was applied to all persons aged 65 years and older who attended an ED between 1986 and 2008. A vertebral fracture was defined using the ICD 10th revision[14]. Vertebral fractures were selected based upon the registered primary diagnosis in the LIS. In case of multiple injuries, the primary injury in LIS was determined by application of an algorithm giving priority to spinal cord injury, skull and brain injury, and lower extremity injury above injuries in other body parts, and to fractures above other types of injury to determine the most serious injury. Numbers of ED visits due to vertebral fractures were specified for age and gender. Furthermore, discharge was registered as treated-and-released or treated-and-admitted to calculate the admission rate. Age-specific rates were calculated in 5-year age groups. The overall age-adjusted incidence rate for the population aged 65 years and older was calculated by using "Direct Standardization" to correct for changes in demographics. Incidence rates were expressed per 100,000 person years. A linear regression analysis was used to analyse the age-adjusted incidence rate of vertebral fracture related ED visits over time. The statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) software (version 16.1.1). A p-value <.05 was considered statistically significant.

RESULTS

From 1986 throughout 2008, the population aged ≥65 years increased from 1.6 million to 2.4 million persons in the Netherlands. During that same period, a total number of 31,650 patients were seen and diagnosed in the ED with a vertebral fracture. The annual number of vertebral fractures requiring ED visits increased with 174% (from 913 in 1986 to 2,502 in 2008), Table 1.

The majority (83%) of vertebral fractures was related to falls in both males and females; this remained unchanged throughout the study period (Table 2).

Table 1. Population characteristics of persons aged ≥65 years, number, incidence, and admissions due to a vertebral fracture (The Netherlands, from 1986 throughout 2008)

Charactaristic	1986	1991	1996	2001	2006	2008
Population ≥65 yr (*1,000)	1,769	1,934	2,061	2,175	2,330	2,415
Population female, %	61.2	60.2	59.8	58.9	57.6	57.0
ED incidence rate†	51.6	56.6	65.9	60.3	83.1	103.6
ED visits, No.	913	1,095	1,358	1,311	1,938	2,502
- Female, No. (%)	634 (69)	789 (72)	981 (72)	816 (62)	1,328 (69)	1,643 (66)
Hospitalization rate†	NA	32.6	36.0	30.0	42.2	57.5
Hospitalized, No. (%)	NA	631 (58)	741 (55)	643 (49)	984 (51)	1389 (55)
- Females, No. (%)	NA	445 (71)	510 (69)	392 (61)	622 (63)	905 (65)

† Crude incidence rate, expressed per 100,000 older adults; NA, not available

Table 2. Causes of vertebral fractures requiring ED attendance between 1986-2008 in older adults aged 65 years and over in the Netherlands

Trauma mechanism	Males		Females		Overall	
	n	%	n	%	n	%
Fall	7,736	78.7%	18,390	84.3%	26,126	82.5%
MVA	1,481	15.1%	1,835	8.4%	3,316	10.5%
Other	610	6.2%	1,598	7.3%	2,208	7.0%
Total	9,826	100%	21,823	100%	31,650	100%

MVA, Motor Vehicle Accident

Gender and age-specific incidence rates are shown in Table 3. Incidence rates increased with ageing and were higher in women than in men for all age groups. The crude incidence rate for men increased from 39.3 per 100,000 older adults in 1986 to 82.7 in 2008 (110% increase). The crude incidence rates for women increased from 59.9 per 100,000 older adults in 1986 to 119.4 in 2008 (99% increase). Figure 1 shows the age-specific incidence rate of vertebral fractures according to 5 year age groups for the periods 1986-1988 and 2006-2008, respectively.

Table 3. Age-specific incidence rates for vertebral fractures related ED visits in persons ≥65 years, per 100,000 older persons (The Netherlands, from 1986 throughout 2008)

	Age-group, year									
Period	65-69		70-74		75-79		80-84		≥85	
	men	women	men	women	men	women	men	women	men	women
1986-1990	33.4	38.8	18.9	60.0	58.6	90.7	53.1	95.3	89.5	88.9
1991-1995	17.2	29.8	25.0	40.0	37.1	62.6	48.3	75.3	123.0	124.4
1996-2000	29.8	54.6	33.5	60.0	47.4	69.5	89.2	125.6	151.8	123.1
2001-2005	35.4	37.1	40.2	58.3	59.7	82.3	73.3	94.9	106.1	140.7
2006-2008	51.5	58.4	61.3	70.8	77.9	112.2	113.8	141.0	216.8	222.8

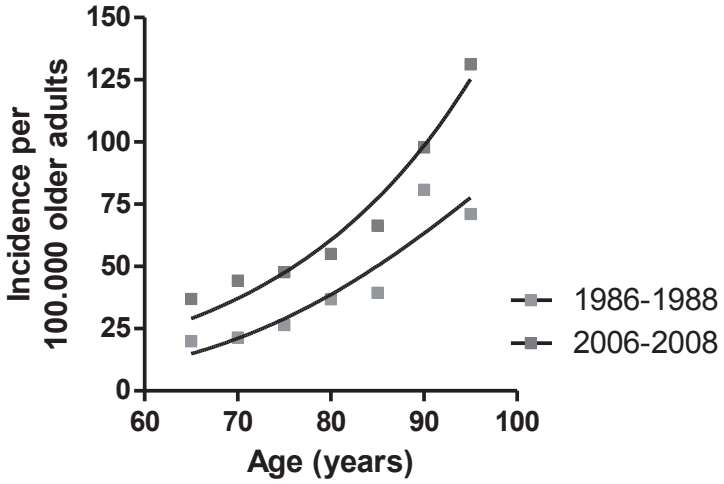

Figure 1. Incidence rate (expressed per 100,000 population) of vertebral fracture related ED visits in the Netherlands per 5 year age-groups; 1986-1988 and 2006-2008

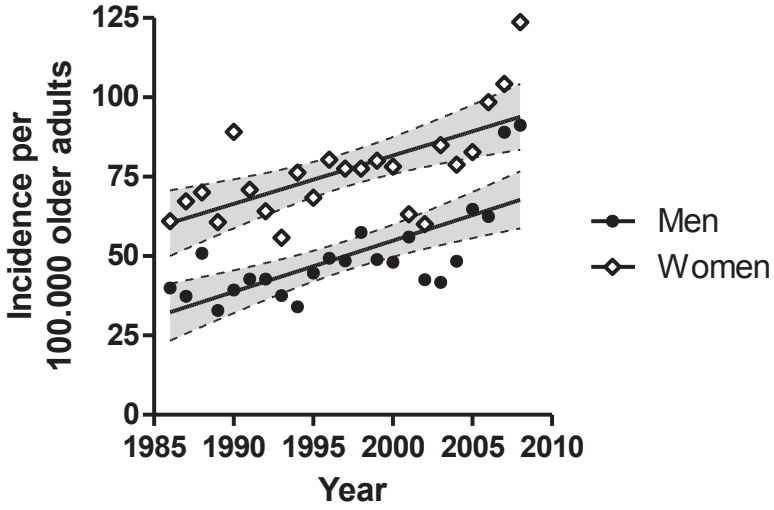

Figure 2. Age-adjusted incidence rate (expressed per 100,000 older persons) of vertebral fracture related ED-visits in persons 65 years and over. The Netherlands, 1986 throughout 2008. The line indicates the age-adjusted incidence rate of ED visits due to a vertebral fracture and the gray area the 95% confidence interval of the linear regression analysis (Trend is significantly different as zero, p<.001 for both lines)

The strongest increase in incidence rate occurred in women ≥85 years, from 88.9 per 100,000 during the period 1986-1990 to 222.8 per 100,000 during the period 2006-2008 (150% increase). The overall age-adjusted incidence rate for ED visits due to vertebral fractures in older adults increased from 51.9

to 102.3 per 100,000 persons (increase 97%) throughout the study period. The age-adjusted incidence rate in women increased from 59.9 per 100,000 population in 1986 to 116.3 in 2008. For men the age-adjusted incidence rate increased from 39.7 in 1986 to 81.2 per 100,000 population in 2008 (Figure 2). During the period 1991-2008 14,658 patients were admitted to the hospital after being diagnosed with a vertebral fracture at the ED. The absolute number of hospital admissions increased from 631 in 1991 to 1,389 in 2008 (120% increase). The overall percentage of patients admitted from the ED during the whole period was 56%. The percentage of hospital admissions did not change over time and remained between 50-55% for both men and women during the study period (Table 1). The adjusted incidence rate of hospital admission after an ED visit for a vertebral fracture increased from 32.6 per 100,000 population in 1991 to 57.1 in 2008. The incidence rate increased most in the age group 85-89 years, from 46.2 per 100,000 population in 1991 to 152.0 in 2008 (229% increase).

DISCUSSION

The aim of this study was to gain insight into secular trends of health care demand due to vertebral fracture related ED visits in the older Dutch population. From 1986 to 2008 the absolute number of vertebral fractures requiring an ED visit increased by 174% to over 2,500 ED visits per year. The age-adjusted incidence rate for ED visits nearly doubled (97% increase) over the last two decades. Especially a strong increase in vertebral fracture related ED visits was seen in individuals aged 80 years and over. The hospital admission rate for people diagnosed with a vertebral fracture at the ED remained fairly constant during the study period at about 55% for both men and women. In over 80% of the cases, the vertebral fracture was related to a fall incident.

Data on clinical-epidemiological characteristics of vertebral fractures are scarce and, as far as we are aware, this is the first study to report on national data regarding vertebral fracture related ED visits and hospital admissions with a study period of over two decades. Some studies with a shorter follow-up have examined incidence rates of vertebral fracture related hospital admissions[18,19]. Vertebral fractures in Spain led to a hospitalization rate of 27.6 per 100,000 population for individuals aged ≥30 years in the year 2002, with a peak of 108.2

per 100,000 population among individuals aged 80 years and over[18]. The Spanish study showed that vertebral fractures affected predominantly women with a female:male ratio of 1.5:1, which is in line with our data. A second study from the United States reported on vertebral fracture related hospital admissions from 1993 throughout 2004. In this study the admission rate increased from 160.9 per 100,000 United States population in 1993 to 180.9 in 2004[19]. In addition, several studies examined the incidence rate of radiographical vertebral fractures in older individuals. In the Rotterdam Study, an ongoing cohort study in over 7,000 older individuals, the incidence of a radiographically diagnosed vertebral fracture in men aged 65-75 years was 5.1 per 1000 person years. In men aged 75 years and over, the incidence rose to 9.3 per 1000 person years. In women, the incidence was 17.0 in those aged 65-75 years and 19.6 per 1000 person years in those aged 75 years and over[20].

In the current study, numbers of fall-related and non-fall related vertebral fractures increased equally as strong. The observed increase in both fall- and non-fall related vertebral fractures might have several causes. An important cause for the observed increase in number of vertebral fractures might be the ageing of the population[13]. Nowadays people live longer, often with multiple medical problems[21]. Ageing and frailty are both risk factors for an increased fall risk and for osteoporosis, and could thus contribute to an increase in vertebral fracture incidence. In the present study, about 80% of the vertebral fractures diagnosed at the ED were fall related. In the literature it has been estimated that about 75% of all vertebral fractures that come to clinical attention are precipitated by routine daily activities such as bending, making beds or lifting (light) objects, and that only 25% of all vertebral fractures in older people is the result of a fall-incident[4]. It can be postulated that fall-related vertebral fractures are over-represented because the ED was taken as intake point in our study. Given the fact that a substantial proportion of the acute hospital care for vertebral fractures is fall-related, it seems plausible that in order to reduce the burden of vertebral fracture related acute admissions, the focus should not only be on the treatment of osteoporosis, but also on the reduction of falls in older persons.

While vertebral fractures are the most common osteoporotic fracture, hip fractures are the second most common osteoporotic fracture, and data on time trends of hip fracture is more readily available. Recent data on secular trends

of incidence rates for hip fractures reported a trend break in incidence rates of hip fracture in the United States[22]. Since 1995, incidence rates of hip fracture started to decline in the American population aged ≥65 years, and similar results are reported in a Canadian study[23]. A similar trend break for vertebral fractures was not found in the current study.

The strength of the present study is the availability of continuous ED monitoring system for an extensive period of 22 years. Throughout the study period, no major policy changes that might have affected the increase in admission rates were introduced in the Netherlands. The Dutch health care system was, and continues to be, characterized by full health insurance coverage and full accessibility for the whole population. Furthermore, the coding system of the LIS did not change during the study period and takes place by official trained coding clerks. A limitation of the use of this linked administrative database is that it does not contain data regarding underlying diagnosis, co-morbidity, treatments or medication use. This hampers the interpretation of causal mechanisms behind the observed trends. Furthermore, readmissions in one calendar year were not excluded and could potentially have led to some "double registrations". However, readmissions have been shown to contribute to only 2.6% of all injury related hospitalizations in the Netherlands[24]. The true burden of vertebral fractures in the older Dutch population will probably exceed the numbers as presented in our study, since only a third of all vertebral fractures are currently diagnosed in clinical practice[25]. This low percentage is partly due to under-diagnosis, especially in the oldest old, and to the atypical presentation of patients with a vertebral fracture[26].

In conclusion, the incidence rate of ED visits in the Netherlands due to a vertebral fracture in persons aged 65 years and over, doubled in the period from 1986 throughout 2008. The increase was most pronounced in the oldest old. A fall was the most frequent cause. This should be a further imperative to a pro-active approach in the diagnosis and treatment of osteoporosis and the prevention of falls in both men and women.

Conflict of interest statement: None declared

Acknowledgements: Klaas Hartholt is a research fellow at the Erasmus MC, appointed on a research grant from "The Netherlands Organization for Health Research and Development" (ZonMw), project number 170.885.607. The funders did not have any influence in study design; in the collection, analysis, and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

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Section 3

Physical and cognitive performance

Chapter 3.1

Vitamin D and physical performance in older men and women visiting the emergency department because of a fall: data from the improving medication prescribing to reduce risk of falls (IMPROveFALL) study.

Nicole D. Boyé, Chirstian Oudshoorn, Nathalie van der Velde, Esther M. van Lieshout, Oscar J. de Vries, Paul Lips, Ed F. van Beeck, Peter Patka, Tischa J.M. van der Cammen

J Am Geriatr Soc. 2013; Nov;61(11):1948-52

ABSTRACT

Objectives: To investigate whether serum 25-hydroxy vitamin D (25(OH)D) is associated with physical performance in men and women.

Design: Cross-sectional.

Setting: Emergency departments (EDs) of five hospitals.

Participants : Older adults who visited an ED because of a fall (N = 616).

Measurements: Physical performance was assessed using the Timed Up and Go Test, the Five Time Sit to Stand Test, handgrip strength, and the tandem stand test. Multivariate linear regression was used to assess the association between physical performance and log-transformed 25(OH)D concentration adjusted for potential confounders.

Results: In men, higher serum 25(OH)D concentration was significantly associated with better handgrip strength (regression coefficient (B) = 3.86, 95% confidence interval (CI) = 2.04 - 5.69), faster TUG time (B = - 2.82, 95% CI = - 4.91 to - 0.73), and faster FTSS time (B = - 3.39, 95% CI = - 5.67 to - 1.11). In women, higher serum 25 (OH)D concentration was significantly associated with faster TUG time (B = - 2.68, 95% CI = - 4.87 to - 0.49).

Conclusion: A positive association was found between serum 25(OH)D level and physical performance in men and women. Intervention studies are needed of vitamin D–deficient older men and women to further investigate the effect of vitamin D supplementation in this group.

INTRODUCTION

Muscle tissue is an important target tissue for vitamin D[1], and vitamin D deficiency is an important contributor to decline in physical performance and increase in fall incidence[2-9], but most studies demonstrating the relationship between serum 25-hydroxyvitamin D_3 (25(OH)D) levels and physical performance have been conducted in female-only populations[5-8]. Studies in men have shown positive or no associations[2,10-12], but most of these studies have been conducted in a population of highly functional, younger men with a low prevalence of vitamin D deficiency. In addition, large studies involving men were population-based, epidemiological studies in randomly selected, nonsymptomatic older persons[2,12] and have not focused specifically on individuals with possible symptoms of neuromuscular dysfunction, such as falls. A fall is generally considered to be a common symptom of neuromuscular dysfunction and vitamin D deficiency[1]. The current study assessed whether serum 25(OH)D was associated with physical performance in community-dwelling older men and women who visited a hospital emergency department (ED) after experiencing a fall.

METHODS

Data collection

Baseline data from the Improving Medication Prescribing to reduce Risk Of Falls (IMPROveFALL) study were used, a detailed description of the methods can be found elsewhere[13]. In short, individuals aged 65 and older who visited the ED because of a fall, used one or more fall–risk increasing drugs, had a Mini-Mental State Examination (MMSE) score of at least 21 of 30 points[14], were able to walk independently, were community dwelling, and provided written informed consent were eligible for enrollment. Enrollment started in October 2008 and was completed in October 2011. The medical ethics committee of the Erasmus MC University Medical Center approved the protocol.

Covariates

A fall was defined as coming to rest unintentionally on the ground or a lower level with or without losing consciousness but not induced by an acute medical condition (e.g., stroke) or an exogenous factor such as a traffic accident[15]. A geriatric assessment was performed at baseline. Medical history, prescription medication, supplements, and lifestyle factors (e.g., education, smoking, alcohol intake) were documented. The number of comorbidities (any malignancy, diabetes mellitus, cardiac disease (hypertension, myocardial infarction, cardiomyopathy, congestive heart failure, arrhythmia, valve disease), chronic obstructive pulmonary disease, stroke, neurological disorders (Parkinson's disease, epilepsy, neuropathy, myopathy, spinal disc herniation, multiple sclerosis), peripheral vascular disease, renal insufficiency, arthritis) was determined. Collected data were verified with records from the participant's general physician and local pharmacist. Height and weight were measured using standardized equipment and procedure. Body mass index (BMI) was calculated as body weight divided by height squared (kg/m^2).

Biochemistry

Nonfasting blood samples were collected at the baseline assessment. Serum 25(OH)D levels were measured using a radioimmunoassay (DiaSorin, Saluggia, Vercelli, Italy). Intra- and interassay coefficients of variation were less than 10%.

Classification of vitamin D status

Serum 25(OH)D groups were chosen based on levels of vitamin D deficiency as described in the literature[1,16]: severe deficiency (<25.0 nmol/L), moderate deficiency (25.0–49.9 nmol/L), sufficient (50.0—74.9 nmol/L), and optimal (≥75.0 nmol/L).

Physical performance

Physical performance was assessed using handgrip strength measurements, the Timed Up and Go (TUG) Test, the Five Time Sit to Stand (FTSS) Test, and the tandem stand test. Handgrip strength[17], was measured in kilograms using a digital strain-gauged dynamometer (Takei TKK 5401, Takei Scientific Instruments Co., Ltd., Tokyo, Japan). Participants were asked to stand upright with

arms hanging beside their body; grip strength was measured in each hand. In the TUG Test[18], the time it took for participants to stand up from a sitting position, walk 3 meters along a line, perform a 180° turn, walk back to the chair, and sit down, as fast as safely possible, was measured. In the FTSS Test[19], the time it took participants to stand up and sit down five consecutive times, as fast as safely possible, was measured. Participants were not permitted to use their hands or the chair's arm supports during standing up or sitting down. In the tandem stand test, participants stood fully independent for 10 seconds with one foot in front of the other. The test was scored as completed[1] or failed (0)[19]. All tests were performed twice, and the best score was recorded.

Statistical analysis

All analyses were performed using the SPSS version 17.0 (SPSS, Inc., Chicago, IL). Baseline characteristics were compared using Student t-test analyses for continuous variables and chi-square analyses for dichotomous variables. Linear regression and binary logistic regression models were constructed to adjust for potential confounders. The crude model was solely age adjusted. Potential confounders considered for inclusion in the multivariate model in addition to age were number of comorbidities, degree of urbanization, marital status, level of education, current or past smoker, alcohol units per day, MMSE score, and BMI. Confounders that led to a change in the regression coefficient (B) of 10% or more were retained in the multivariate-adjusted regression model. Participants with incomplete or missing performance test measures were excluded from related analyses (handgrip strength, n = 7; TUG Test, n = 55; FTSS Test, n = 95; tandem stand test, n = 4). Measures were missing mostly because of injuries after the fall (e.g., upper or lower extremity fractures) or preexisting conditions. Because of a right-skewed distribution, serum 25 (OH)D levels were log transformed (natural log) for the regression models, and a general linear model was used to multivariately compare all continuous outcomes and chi-square analyses to compare the tandem stand outcomes. All analyses were stratified according to sex, and $P < .05$ was considered statistically significant.

RESULTS

Six hundred sixteen participants were enrolled in the IMPROveFALL study. Information on serum 25(OH)D concentration was obtained from 600 participants (230 (38%) men; 370 (62%) women). The sex-specific baseline characteristics are shown in Table 1; the mean age was 76 ± 7.

Table 1. Baseline characteristics according to sex

Characteristic	Men (n=230)	Women (n=370)	*p*-value
Age, mean ± SD	76.4 ± 6.7	76.5 ± 7.0	0.820
Serum 25(OH)D, mean ± SD	58.9 ± 30.9	58.7 ± 27.8	0.939
MMSE score, mean ± SD	27.0 ± 2.3	26.9 ± 2.4	0.716
Body mass index (kg/m^2), mean ± SD	27.1 ± 3.9	27.9 ± 4.9	0.027
Secondary level of education, n (%)	185 (80)	250 (68)	<0.001
Urban, n (%)	190 (83)	323 (87)	0.142
Recurrent fallers, n (%)	96 (42)	178 (48)	0.128
Smoking, n (%)			
Current	28 (12)	40 (11)	0.609
Past	152 (66)	122 (33)	<0.001
Never	76 (33)	245 (66)	<0.001
Alcohol units p/day, n (%)			<0.001
0	92 (40)	212 (57)	
<1	24 (10)	63 (17)	
1-3	66 (29)	78 (21)	
>3	48 (21)	17 (5)	
Vitamin D supplements, n (%)	14 (6)	61 (17)	<0.001
Number of comorbidities, mean ± SD	2.1 ± 1.2	2.1 ± 1.2	0.654
Number of medications, mean ± SD	5.9 ± 2.9	6.5 ± 3.5	0.027
Number of FRIDs, mean ± SD	2.5 ± 1.5	2.7 ± 1.6	0.378

FRID: fall-risk increasing drugs.

The mean serum 25(OH)D concentration was 59 ± 29 nmol/L. Fifty-five participants (9%) had severe vitamin D deficiency (<25 nmol/L), 209 (35%) had moderate deficiency (25—49.9 nmol/L), 172 (29%) had sufficient vitamin D (50—74.9 nmol/L), and 164 (27%) had optimal 25(OH)D levels (≥75 nmol/L). One hundred two men (44%) and 162 women (44%) had serum 25(OH)D levels of less than 50 nmol/L.

Regression models of physical performance according to log-transformed serum 25(OH)D concentration were constructed (Table 2). In men, in the fully adjusted model, higher serum 25(OH)D concentrations were significantly associated with better handgrip strength (B = 3.86, 95% CI = 2.04—5.69), faster TUG times (B = −2.82, 95% CI = −4.91 to −0.73), and faster FTSS times (B = −3.39, 95% CI = −5.67 to −1.11). In women, higher serum 25(OH)D concentrations were significantly associated with faster TUG times (B = −2.68, 95% CI = −4.87 to −0.49).

Table 2. Results of regression analysis of strength and physical performance according to log transformed serum 25 (OH)D concentration and sex

	Model 1	Model 2
Men (n = 230)		
Handgrip strength (n=228)	4.02 [2.30; 5.75]***	3.86 [2.04; 5.69]***
Timed "Up & Go" (n=211)	-3.02 [-5.03; -1.02]**	-2.82 [-4.91; -0.73]**
Five Time Sit to Stand (n=197)	-3.11 [-5.27; -0.94]**	-3.39 [-5.67; -1.11]**
Tandem stand (n=230)	0.59 [1.05; 3.11]*	0.55 [0.93; 3.19]
Women (n = 370)		
Handgrip strength (n=365)	0.80 [-0.13; 1.72]	0.67 [-0.26; 1.61]
Timed "Up & Go" (n=334)	-3.19 [-5.34; -1.04]**	-2.68 [-4.87; -0.49]*
Five Time Sit to Stand (n=308)	-2.69 [-4.90; -0.49]*	-2.13 [-4.30; 0.04]
Tandem stand (n=366)	0.15 [0.77; 1.76]	0.04 [0.68; 1.59]

Data are shown as B with the 95% confidence interval between square brackets.
Model 1: adjusted for age. Model 2: adjusted for age, number of comorbidities, smoking, degree of urbanization, body mass index, and MMSE score. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

A general linear model was used to compare the means of handgrip strength, TUG, and FTSS (Figure 1 A–C) according to sex and vitamin D group. In men, handgrip strength ranged from 30.3 kg (in participants with serum 25(OH)D levels <25 nmol/L) to 36.0 kg (in participants with 25(OH)D levels >75 nmol/L) (21.0 and 21.6 kg, respectively, for women). Results for the TUG Test ranged from 12.1 seconds (serum 25(OH) <25 nmol/L) to 9.9 seconds (serum 25(OH) D) > 75 nmol/L) in men; results in women were 18.8 seconds and 12.0 seconds, respectively. Results for the FTSS Test ranged from 20.0 seconds (serum 25(OH) <25 nmol/L) to 14.6 seconds (serum 25(OH)D) > 75 nmol/L) in men; results in women were: 23.3 seconds and 17.9 seconds, respectively. The percentage of completed tandem stands according to vitamin D group was 68%, 59%, 64%, and 86%, respectively, in men (P = .009) and 44%, 61%, 66%, and 63%, respectively, in women (P = .15).

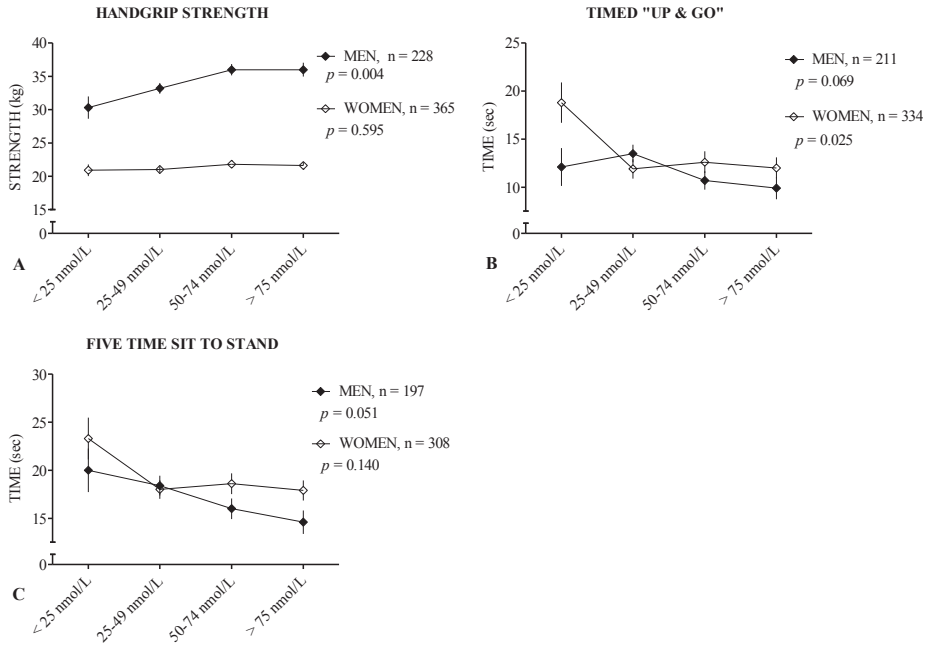

Figure 1. Strength and physical performance according to serum vitamin D group and sex. General linear model analysis of (A) handgrip strength, (B) Timed Up and Go Test, and (C) Five Time Sit to Stand test with mean and standard error. Adjusted for age, number of comorbidities, smoking, degree of urbanization, body mass index, and Mini Mental State Examination score

DISCUSSION

Serum 25(OH)D levels were significantly associated with physical performance in older fallers who visited a hospital ED after a fall. The population consisted of older men and women with a mean age of 76, many of whom were vitamin D deficient; an average of 44% had 25(OH)D levels less than 50 nmol/L, making it a good population in which to investigate the relationship between vitamin D and physical performance.

In men, an association was found between serum 25(OH)D levels and handgrip strength, TUG time, and FTSS time. In women, there was an association only between serum 25(OH)D level and TUG time. Various studies have demonstrated a relationship between vitamin D and physical performance[5-11,20,21]. Although most of these studies were conducted in female-only populations[5-8], some studies that included men and women reported similar results[2,20]. In

addition, a 3-year follow-up study reported poorer physical performance and a greater decline in physical performance in older vitamin D–deficient men and women[9], but recent studies investigating men specifically have not found an association between vitamin D levels and physical performance[10, 11]. Lack of an association in the previously mentioned studies may be because of the target population (young, healthy men) and the low prevalence of vitamin D deficiency. In the current study, older persons were included who visited the hospital after a fall, which can be a sign of possible neuromuscular dysfunction. Variations in baseline functional status and vitamin D status are thought to be important in explaining conflicting results in studies that examine the association between vitamin D and muscle function[22].

The different results found for men and women, especially in the FTSS, are not easily explained. It is unclear whether vitamin D deficiency affects men and women differently. There is some evidence, mostly from ex vivo and in vitro studies, that this is the case[23]. Sex hormones are known to modulate the vitamin D endocrine system and influence calcium homeostasis. For example, estrogen is known to stimulate vitamin D receptor expression and 1α-hydroxylase activity, and testosterone is reported to stimulate intestinal calcium channel expression[23].

The following limitations should be taken into account when interpreting the results of the current study. First, the cross-sectional design of the study limits the ability to infer a causal relationship between serum 25(OH)D levels and physical performance and does not exclude the possibility of reverse causality. Second, serum parathyroid hormone (PTH) levels were not determined. Vitamin D deficiency leads to an increase in serum PTH, which increases bone turnover and bone loss and is related to a decrease in muscle strength[23]. Third, the use of a MMSE score of less than 21 as an exclusion criterion could have resulted in the exclusion of the frailest persons. Fourth, information regarding mood or depression was not recorded. Mood disorders might affect vitamin D status and neuromuscular performance. A major strength of this study is the substantial proportion of vitamin D–deficient participants included, which enabled physical performance to be analyzed in vitamin D–deficient and –sufficient men and women.

In addition, it was striking to note how few of the older fallers were prescribed vitamin D supplements, especially the men; although an average of 44% of the men and women were deficient in vitamin D, only 6% of the men and 17% of the

women were taking vitamin D supplements. The underprescribing of vitamin D in this age group has been reported[24], but despite evidence that vitamin D supplementation has been shown to increase muscle strength and reduce the risk of falls[25], vitamin D deficiency is common in community-dwelling elderly adults, with a prevalence of 40% to 100% in U.S. and European older men and women[1]. Furthermore, although the level for vitamin D sufficiency was set at 50 nmol/L or greater, another opinion is that vitamin D levels should be 75 nmol/L or greater[26]. This is interesting to note when considering Figure 1, which indicates that levels closer to 75 nmol/L result in physical performance benefits, especially in men.

In conclusion, higher serum 25(OH)D concentration was associated with better strength and physical performance in older male and female fallers.

Acknowledgements: We thank Eunice Comvalius, medical student, for her assistance with data collection and manuscript preparation.

Conflict of interest: The editor in chief has reviewed the conflict of interest checklist provided by the authors and has determined that the authors have no financial or any other kind of personal conflicts with this paper.

This work was supported by research grant 170.885.607 from the Netherlands Organization for Health Research and Development.

Author contributions: Boyé: study design, enrollment of participants, acquisition, analysis and interpretation of data, preparation of manuscript. Oudshoorn: analysis and interpretation of data, preparation of manuscript. Van der Velde: study design, enrollment of participants, analysis and interpretation of data, preparation of manuscript. Van Lieshout, Van Beeck, Patka: study design, interpretation of data, and preparation of manuscript. De Vries, Lips: enrollment of participants, acquisition of data, revision of manuscript. Van der Cammen: study design, enrollment of participants, interpretation of data, revision of manuscript. All authors approved the final version of the manuscript.

Sponsor's role: None.

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Chapter 3.2

Effect of high dose vitamin D supplementation on neuromuscular function in older vitamin D deficient individuals living in residential care: a double blind placebo controlled trial

C. Oudshoorn, E.M. Colin, N. van der Velde, J.P.T.M. van Leeuwen, T.J.M. van der Cammen

Submitted

ABSTRACT

Background: Vitamin D deficiency is a prevalent condition among older individuals, and is associated with myopathy and a decrease in neuromuscular performance.

Objective: To study the effects of a daily dose of 1800 IU vitamin D_3 on muscle strength, gait speed and Timed Up and Go Test (TUGT) performance.

Design: In this double-blind trial, subjects aged ≥ 65 years, living in residential homes, with serum 25-hydroxyvitamin D_3 ($25(OH)D_3$) concentrations < 50 nmol/l were randomly assigned to receive either a daily dose of 1800 IU vitamin D_3 (n = 124) or placebo (n = 118).

Endpoints: Change in quadriceps extension strength was the primary endpoint. Secondary endpoints included change in handgrip strength, 6 meter walking test performance and Timed Up and Go Test performance.

Results: Serum $25(OH)D_3$ concentrations rose significantly (from 32.1 nmol/l to 107.3 nmol/l, $P < 0.001$) in participants treated with a daily dose of 1800 IU vitamin D_3. An improvement in gait speed and TUGT performance was observed in the intervention group of respectively 6.0% and 4.8%. The improvement was most pronounced in the participants with baseline $25(OH)D_3$ levels of ≤ 25 nmol/l. No effect on quadriceps strength or handgrip strength was observed.

Conclusions: Daily treatment with 1800 IU vitamin D_3 raised $25(OH)D_3$ concentrations in older, mild-moderate vitamin D–deficient individuals was associated with an improvement in gait speed and TUGT performance. Further studies with a longer follow up are needed to assess the additional benefit and safety of high dose supplementation. This trial is registered at Current Controlled Trials as ISRCTN33988275.

INTRODUCTION

Vitamin D, a seco-steroid hormone, is mostly known because of its importance for calcium homeostasis and bone health. In recent years it has become clear that the vitamin D receptor (VDR) via which the vitamin D metabolites exert their function, is expressed by almost all cells in the human body, including skeletal muscle cells[1]. Via the VDR, the vitamin D metabolites exert both genomic and non-genomic effects on muscle cells which are important for muscle function[2]. Older people are at particular risk of developing a vitamin D deficiency because of a decreased cutaneous vitamin D production capacity, a decrease in time spent outdoors, and less dietary intake of vitamin D[3].

Key symptoms of vitamin D deficiency associated myopathy are proximal muscle weakness, muscle pain and gait disturbances[4]. Observational studies have reported numerous associations between vitamin D status and neuromuscular function in older individuals. Intervention trials assessing the effect of vitamin D supplementation on neuromuscular performance have yielded inconsistent results[5, 6]. Although various methodological causes can be considered, an important cause of several negative trial results could be that the dose of vitamin D supplementation used is too low, i.e. the obtained serum 25(OH)D_3 level after the intervention is too low. Large observational studies suggest that optimal 25(OH)D_3 levels for neuromuscular performance could be as high as > 75 – 100 nmol/l, a level which is not reached in most intervention trials[7]. Therefore we assessed the effect of high dose vitamin D supplementation (1800 IU/day) on neuromuscular function in older people and hypothesized that high dose vitamin D supplementation has a more pronounced effect on muscle strength and mobility compared to low dose vitamin D supplementation.

METHODS

Subjects

Participants were recruited from residential homes during the period March 2008 – March 2010. Participants were eligible if age was ≥ 65 years, they were in stable health, they were able to perform (most of) the neuromuscular tests and serum 25(OH)D_3 level was < 50 nmol/l. Exclusion criteria were: renal insufficiency

(GFR < 40 ml/min), hypercalcaemia (serum calcium > 2.70 nmol/l), diagnosis of dementia or mini mental state examination score < 20 points, primary hyperparathyroidism, current malignant disease (any), history of renal stones and use of digoxin or anti-epileptic drugs (any). Participants were also excluded if they had a history of recurrent falls. The initial selection process was performed by the staff of the residential care facility, based on both the inclusion and exclusion criteria, expected willingness to participate and chance of finalizing the follow up period. The protocol was approved by the Medical Ethics Committee of the Erasmus MC (MEC 2007-160) and written informed consent was obtained from all participants.

Study design

This 6-month randomized, double-blind, placebo-controlled, trail, to assess efficacy and safety of daily 1800 IU vitamin D_3 in older vitamin D deficient individuals was conducted in residential homes in the western part of the Netherlands. The primary endpoint was change in quadriceps strength during the study period. Secondary endpoints included change in handgrip strength, gait speed and mean serum 25(OH)D_3 level. Participants were randomized to receive either a daily dose of 1800 IU vitamin D_3 (cholecalciferol) or a placebo. Trial medication consisted of two vials of 100ml each, with a vitamin D solution of 1800 IU/ml or matching placebo. Compliance was monitored by staff in the residential homes and documented. Adverse events (AEs) were recorded by the staff of the residential home at any time during the study.

Baseline characteristics

Participants were selected based on medical history, current physical condition, and medication use including supplements. Information total morbidity was obtained from the nurses' record of the residential home. Cognitive functioning was assessed using the Dutch version of the Mini-Mental State Examination (MMSE), with a score ranging from 0 points (poor cognitive functioning) to 30 points (good cognitive functioning).

Assessment of muscle strength and neuromuscular function

Four different tests were performed, namely: isometric quadriceps femoris muscle extension strength measurement, handgrip strength measurement, 6-meter walking test and Timed Up and Go Test (TUGT) performance. For each test, it was first checked whether the patient knew how to perform the test properly; handgrip strength and quadriceps strength measurement were rehearsed one time. All tests were performed two times. The best score of the two measurements was used for the analysis. Maximal isometric quadriceps femoris muscle extension strength was measured preferably at the dominant side with the handheld MicroFET dynamometer (in Newton). During this measurement the dynamometer is placed just proximal to the ankle on the front lower leg (on the distal tibia and anterior tibial muscle tendon). The researcher then holds the dynamometer stationary while the participant exerts a maximal force against it; therefore the test is essentially isometric. Handgrip strength (in Kg) at the dominant side was measured using a portable Takei handheld dynamometer (Takei Scientific Instruments Co. Ltd, Tokyo, Japan). The 6-meter walking test measures the time (in seconds) that it takes a patient to walk 6 meters. It assesses short-duration walking speed. The TUGT is a test for balance that is commonly used to examine functional mobility in older adults. The test requires a subject to stand up, walk 3 meter, turn, walk back, and sit down. The duration of this test is measured in seconds. All measurements were performed at baseline and after six months. In case participants completed taking the trial medication before the end of the 6 month follow up period, follow up measurements were performed earlier.

Vitamin D measurement

Laboratory measurements were performed at the Erasmus University Medical Center. In case of logistic problems measurement were performed by the local laboratory, affiliated with the particular residential care facility. In some cases, where serum $25OHD_3$ level measurement had been recently performed (< 1 month) by the general practitioner, this measurement was used as baseline measurement (measurement also performed by local laboratory). In the Erasmus University Medical Center, serum $25(OH)D_3$ levels (in nmol/l) were measured using a radio-immuno-assay (DiaSorin, Stillwater, MN, USA).

Statistical methods

Before starting the study, we estimated that for our main study outcome regarding the effect of vitamin D supplementation we would need 250 participants to have 80% power for a detection of an improvement of 7%, taking into account an alpha of 0.05.

Population characteristics were reported as mean ± SD. Baseline differences between the two groups (intervention or placebo) were tested using an independent t-test for normally distributed variables (age, gender and MMSE score) and the Mann–Whitney test for skewed variables (calcium intake and serum 25(OH)D3 level, MMSE score).

Within-group changes over the 6 month study period in the intervention and the placebo groups were compared using a t-test for paired observations. Statistical significance of the difference in change between the intervention and the placebo group were tested using an independent sample t-test. Data were analyzed on an intention to treat basis. Missing values were imputated by random sampling using the mean and standard deviation.

All statistical analyses were performed using SPSS software (version 16.1.1; SPSS Inc., Chicago, Illinois). A p-value of < 0.05 was considered statistically significant.

RESULTS

In this prospective, randomized, double-blind, placebo controlled trial, 409 subjects who fulfilled the inclusion criteria were screened for vitamin D deficiency (Figure 1). Of those, 242 were randomized to receive either vitamin D supplementation or placebo. The main reasons for exclusion were memory complaints or a too low MMSE score, and withdrawal of informed consent.

The characteristics of the study participants are shown in Table 1. The mean age of the study participants was 81.2 ± 6.9 years with a mean serum 25(OH) D_3 level of 32.5 ± 7.7 nmol/l. At baseline there were no differences between the two treatment groups. During the study period, 3 participants died and 16 were lost to follow up. Tolerability of the trial medication was good; no specific drug related complaints were reported.

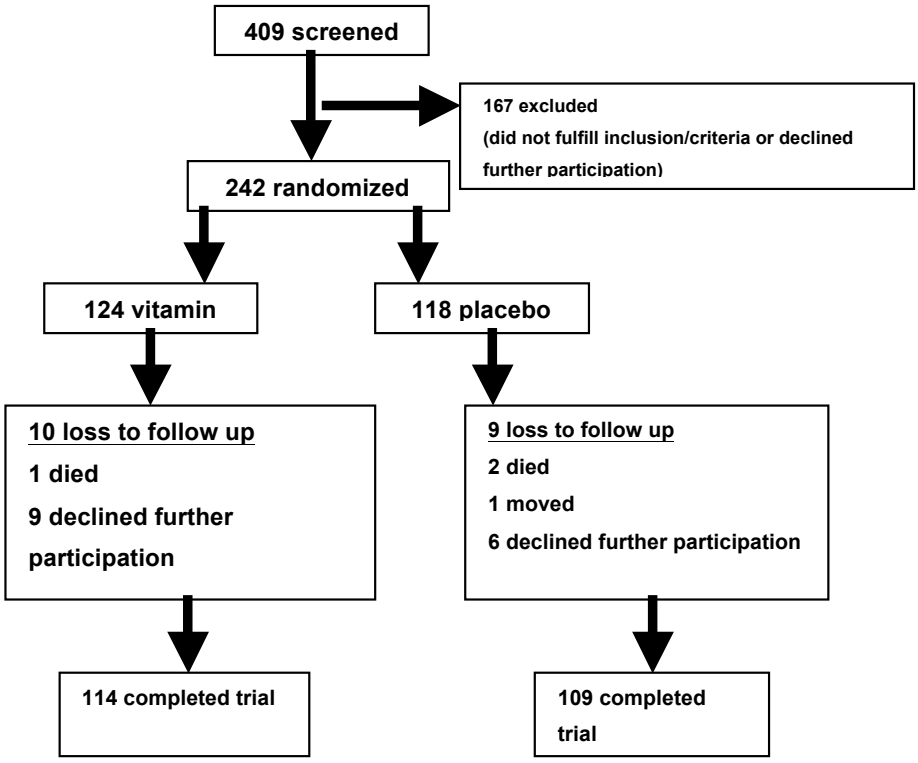

Figure 1 Study flowchart

Table 1 Baseline characteristics of the study participants

Characteristic	Vitamin D_3 (n = 124)	Placebo (n = 118)	P value
Age, yr	81.5 ± 7.0	80.6 ± 6.7	p = 0.32
Female, n (%)	94 (76%)	85 (72%)	P = 0.51
MMSE score (points)	26.8± 2.5	26.4 ± 2.6	p = 0.30
Serum 25(OH)D_3 (nmol/l)	32.1 ± 8.5	33.3 ± 7.1	p = 0.21
Serum calcium (mmol/l)	2.28 ± 0.10	2.26 ± 0.11	p = 0.16
Serum creatinine (µmol/l)	75.8 ± 12.1	73.1 ± 12.5	p = 0.09
Quadriceps extension strength (N)	163.1 ± 29.5	166.9 ± 29.4	p = 0.31
Handgrip strength (Kg)	23.8 ± 4.0	24.1 ± 4.2	p = 0.30
6m walking test (s)	6.7 ± 1.5	6.5 ± 1.5	p = 0.43
Timed Up and Go Test (s)	12.5 ± 2.4	12.2 ± 2.2	P = 0.40

Effects on vitamin D status and serum calcium levels

The dose of vitamin D was effective in increasing mean 25(OH)D_3 levels at 6 months compared with placebo. During the study period mean 25(OH)D_3 level in the placebo group increased from 33.3 to 34.5 nmol/l (3.6% increase; p = 0.06). In the active treatment group, mean 25(OH)D_3 levels increased from 32.1 to 107.3 nmol/l (234.1 % increase; p < 0.001).

Effect on muscle strength

Table 2 shows the changes in muscle strength for the intervention and placebo group. Overall quadriceps strength in the intervention group improved from 163N to 165N (p > 0.05). No association was seen with baseline serum 25(OH)D_3 level. In the placebo group, also significant change was seen in muscle strength. Mean handgrip strength in the intervention group increased from 23.8 Kg to 24.2 Kg (p > 0.05). Again, no association was seen with baseline serum 25(OH) D_3 level. Also, in the placebo group, no significant change was seen in handgrip strength. For both quadriceps strength and handgrip strength no association correlation was seen between change in vitamin D status and change in vitamin D status during the intervention (p >0.05)

Table 2 Change in muscle strength

Test	Vitamin D (n = 124)				Placebo (n = 118)			
		Baseline	Follow Up	% change		Baseline	Follow up	% change
Quadriceps strength (N)								
Overall	(n = 124)	163.1 ± 29.5	165.4 ± 30.8	1.4%	(n =118)	166.9 ± 29.4	167.7 ± 29.2	0.5%
s-25(OH)D_3 ≤ 25	(n = 31)	156.6 ± 28.4	158.5 ± 28.6	1.2%	(n=15)	161.7 ± 25.9	160.6 ± 24.0	- 0.7%
s-25(OH)D_3 > 25	(n = 93)	165.2 ± 29.7	167.8 ± 31.4	1.6%	(n = 103)	167.6 ± 29.9	168.7 ± 29.8	0.6%
Handgrip strength (Kg)								
Overall	(n = 124)	23.8 ± 4.0	24.2 ± 4.1	1.7%	(n = 118)	24.1 ± 4.2	24.3 ± 3.9	0.8%
s-25(OH)D_3 ≤ 25	(n = 31)	23.1 ± 4.1	23.8 ± 4.5	3.0%	(n=15)	23.1 ± 3.8	23.2 ± 3.9	0.4%
s-25(OH)D_3 > 25	(n = 93)	24.0 ± 3.9	24.3 ± 4.0	1.3%	(n = 103)	24.3 ± 4.3	24.4 ± 3.9	0.4%

* t-test for paired observations, significantly different from baseline, P < .05

Effect on neuromuscular performance

Table 3 shows the changes in neuromuscular test performance for the intervention and placebo group. In the intervention group there was a significant improvement in gait speed. Mean 6 meter walking time decreased from 6.7 seconds (equals 0.90 m/s) to 6.3 seconds (equals 0.95 m/s, 6.0% improvement; $p < 0.05$). In participants with baseline 25(OH)D_3 levels of ≤ 25 nmol/l, 6 meter walking time improved 8.3% versus 4.6% in participants with serum 25(OH)D_3 levels > 25 nmol/l ($p < 0.05$). No significant change was seen in gait speed in the placebo group. TUGT performance in the intervention group improved with 4.8%. The improvement in participants with a baseline serum 25(OH)D_3 level of ≤ 25 nmol/l was 7.7% ($p < 0.05$). The improvement in participants with a baseline 25(OH)D_3 level > 25 nmol/l was 3.4% ($p > 0.05$). For both 6 meter walking test and TUGT, no correlation was observed between change in test performance and change in vitamin D status during the intervention ($p > 0.05$).

Table 3 Change in gait speed and Timed Up and Go Test (TUGT) performance

Test		Vitamin D (n = 124)				Placebo (n = 118)		
		Baseline	Follow Up	% Change		Baseline	Follow up	% Change
Gait speed (s)								
Overall	(n = 124)	6.7 ± 1.5 (0.90 m/s)	6.3 ± 1.3 (0.95 m/s)	- 6.0%*	(n =118)	6.5 ± 1.5 (0.92 m/s)	6.7 ± 1.4 (0.90 m/s)	3.1%
s-25(OH)D_3 ≤ 25	(n = 31)	7.2 ± 1.5	6.6 ± 1.2	- 8.3%*	(n=15)	7.5 ± 1.6	7.4 ± 1.3	- 1.3%
s-25(OH)D_3 > 25	(n = 93)	6.5 ± 1.4	6.2 ± 1.3	- 4.6%*	(n = 103)	6.4 ± 1.5	6.5 ± 1.4	1.6%
TUGT (s)								
Overall	(n = 124)	12.5 ± 2.4	11.9 ± 2.4	- 4.8%*	(n =118)	12.2 ± 2.2	12.4 ± 2.2	1.6%
s-25(OH)D_3 ≤ 25	(n = 31)	13.0 ± 2.3	12.0 ± 2.0	- 7.7%*	(n=15)	12.6 ± 2.1	12.9 ± 2.0	2.4%
s-25(OH)D_3 > 25	(n = 93)	12.3 ± 2.4	11.9 ± 2.5	- 3.4%	(n = 103)	12.2 ± 2.3	12.3 ± 2.2	0.8%

* t-test for paired observations, significantly different from baseline, P < .05

DISCUSSION

In the present study, performed in older, vitamin D deficient individuals, living in residential care, treatment with high dose cholecalciferol for 6 months improved both gait speed and TUGT performance. Improvement was most pronounced in participants with baseline serum 25(OH)D_3 levels ≤ 25 nmol/l.

No effect of vitamin D supplementation was observed on quadriceps extension strength and handgrip strength.

A daily dose of 1800 IU vitamin D_3 was effective in raising the mean serum 25(OH)D_3 concentration from a mean of 32.1 to 107.3 nmol/l in the intervention group. In the placebo group, serum 25OHD_3 levels did not change significantly, suggesting marginal seasonal influences on vitamin D status in this cohort. The high dose vitamin D supplementation was well tolerated in this cohort of older people and no adverse events were reported.

The results of the present study support the hypothesis that vitamin D is important for muscle function, and the results suggest that a state of vitamin D deficient myopathy is potentially reversible with vitamin D supplementation. Previous studies showed variable results regarding the effect of vitamin D supplementation on muscle strength and neuromuscular performance. In this study, no effect of high dose vitamin D supplementation was observed on either quadriceps strength and handgrip strength. These findings contrast with some previous results that did report an effect on muscle strength. For example, a previous study in frail older women showed that a supplementation dose of 1000 IU ergocalciferol for 2 years resulted in an increase of quadriceps strength of almost 50%[8]. In two other studies vitamin D supplementation resulted in a 4% to 11% improvement in lower extremity strength or function. On the other hand for example, studies by Brunner et al. and Dhesi et al., failed to find an effect of vitamin D supplementation on muscle strength[9, 10]. Possible causes of these variable study results are thought to be variation in supplementation duration and dose, variation in participant age and co-morbidities. In addition, a recent meta-analysis suggested that severity of vitamin D deficiency could be important. This study concluded that vitamin D supplementation does not have a significant effect on muscle strength in adults with baseline 25OHD_3 levels > 25 nmol/l[11].

With regard to the effect of vitamin D supplementation on gait speed, the observed trial results in the literature are also variable. In this study an overall improvement in gait speed of 6.0% was observed, and this improvement was 8.3% in participants with baseline 25OHD_3 levels < 25 nmol/l versus 4.3% in participants with baseline levels > 25 nmol/l. Parallel to our findings, a study by Bunout et al., reported an improvement of gait speed with a vitamin D

supplementation dose of 400IU/day and 800mg of calcium/day for 9 months[12]. These findings contrast several other studies that found no effect of vitamin D supplementation on gait speed[10, 13, 14]. Again, these studies varied strongly in supplementation dose, study population and trial duration. In a recent meta-analyses by Muir et al., an effect on gait speed was not found. This study concluded that most trials had methodological issues that limited comparability, and further research was recommended[15]. A recent study by Bischoff-Ferrari et al., examining the effect of vitamin D supplementation on gait speed, demonstrated a superior effect of calcifediol versus cholecalciferol on gait speed. In this study, women receiving calcifediol had an 18% greater improvement in gait speed at 4 months follow up, compared to women receiving cholecalciferol supplementation[16].

In the present trial, high dose vitamin D supplementation was used, which resulted in a mean serum $25OHD_3$ level of 107 nmol/l in the intervention group. No correlation however was observed between change in serum $25(OH)D_3$ level and improvement in neuromuscular function or muscle strength; i.e. a greater improvement in vitamin D status did not result in a greater improvement in test performance. This raises the question what the ideal supplementation dose or serum $25OHD_3$ level is, and if high dose supplementation is truly beneficial. Furthermore, the observed improvement in this trial, when compared to other positive trials, that used lower dose supplementation, is not superior[17]. Furthermore, in regard to the observed improvement in gait speed, the clinical relevance is unclear. The mean observed improvement was 0.05m/s, while generally a change of at least 0.1 m/s in gait speed is considered a substantial meaningful change[18, 19]. The issue of defining the optimal serum $25OHD_3$ level is relevant because, while no adverse effects were observed in this relatively short trial, some observational studies on cardiovascular endpoints reported an U-shaped risk profile between cardiovascular risk and serum $25(OH)D_3$ levels[20-22]. The nadir in most of these studies with regard to the cardiovascular risk or survival was about 60 nmol/l. An increase in fall and fracture risk with high dose vitamin D supplementation has also been reported[23, 24].

This trial has several strengths. Given the fact that medication was administered by the staff of the residential care facilities, compliance was well documented. Furthermore, loss to follow up in this trial was limited. However,

several limitations have to be considered. First, given the fact that this trial was performed in residential care facilities, results can not readily be extrapolated to community dwelling older persons. Second, in this trial we used a liquid vitamin D supplement. This may have resulted in dosing problems and some variation in daily dose. Many participants finished the vials of trial medication before the intervention period of 6 months had passed, which presumably resulted in a higher net intervention dose than 1800 IU/day. Third, serum $25OHD_3$ measurements were performed in various laboratories while it is known that measurement results vary between laboratories and methods. However, given the fact that this occurred in both the intervention and the control group, it is not likely that this affected the outcome of this trial. Fourth, no information was available on body mass index (BMI) while it is known that $25(OH)D_3$ levels are affected by BMI given the fact that it is a fat soluble hormone.

In conclusion, high dose vitamin D supplementation improved gait speed and TUGT performance in older persons living in residential care. No effect was observed on muscle strength. Future well designed studies with longer follow up periods are needed to further assess the effect of vitamin D supplementation on skeletal muscle function, and to define the optimal serum $25OHD_3$ level for muscle function.

ACKNOWLEDGMENT

We would like to thank B. Li for statistical advice.

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Chapter 3.3

Higher serum vitamin D_3 levels are associated with better cognitive test performance in patients with Alzheimer's disease

Christian Oudshoorn, Francesco U.S. Mattace-Raso, Nathalie van der Velde, Edgar M. Colin and Tischa J.M. van der Cammen

Dement Geriatr Cogn Disord. 2008; 25(6):539-43

ABSTRACT

Background/Aims: Recent studies suggest that vitamin D metabolites may be important for preserving cognitive function via specific neuroprotective effects. No large studies have examined the association between vitamin D status and cognition.

Methods: In this cross-sectional study, we analyzed the serum 25-hydroxyvitamin D_3 levels and Mini-Mental State Examination (MMSE) test scores of 225 older outpatients who were diagnosed as having probable Alzheimer's disease (AD). In addition to the 25-hydroxyvitamin D_3 levels, we analyzed the serum vitamin B_1, B_6 and B_{12} levels.

Results: An association was found between MMSE test scores and serum 25-hydroxyvitamin D_3 levels, with β-coefficient of 0.05 ($p = 0.01$). Vitamin-D-sufficient patients had significantly higher MMSE scores as compared to vitamin-D insufficient ones. No association was found with the other serum vitamin levels.

Conclusions: These data support the idea that a relationship exists between vitamin D status and cognition in patients with probable AD. However, given the cross-sectional design of this study, no causality can be concluded. Further prospective studies are needed to specify the contribution of vitamin D status to the onset and course of cognitive decline and AD.

INTRODUCTION

Dementia is associated with a higher rate of comorbidity, poor functional status and increased mortality[1–3]. The emotional and financial burden of dementia and Alzheimer's disease (AD) on society will increase in the next decades[4]. The clinical assessment of dementia in specialized centers, such as memory clinics, has led to earlier diagnosis and has stimulated the search for potential therapeutic agents and preventative strategies[5]. The American Academy of Neurology recommends screening for vitamin B_{12} deficiency and hypothyroidism in patients with dementia[6]. In the Dutch consensus, screening for folate and vitamin B_1 , B_6 and B_{12} deficiency is recommended[7].

Recent insights suggest that vitamin D, mostly known for its effects on calcium and bone metabolism, may have neuroprotective functions and could be important for preserving cognitive functions via several different mechanisms[8].

Vitamin D is a secosteroid hormone. The term vitamin D refers to the inert precursors vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol). The precursors are either formed in the skin after exposure to sunlight or derived from dietary sources. In the liver, the precursors are converted to 25-hydroxyvitamin D_3 [25-(OH)D_3]. In the kidney, 25-(OH)D_3 is hydroxylated to 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$], the most active vitamin D metabolite [9]. The serum 25-(OH)D_3 levels are generally used to determine the vitamin D status of an individual. Serum 25-(OH)D_3 levels of ≥ 50 nmol/l are generally defined as sufficient, although recent reports suggest that a serum 25-(OH)D_3 level ≥ 75 nmol/l may be preferable for optimal health[10].

The vitamin D metabolites exert their effects via the vitamin D receptor (VDR). Recently, it has been shown that neurons also express the VDR, which makes them a potential target tissue for vitamin D metabolites[11]. The VDR is abundantly expressed in regions frequently affected in AD such as the hypothalamus, substantia nigra, cortex and hippocampus. The importance of a sufficient vitamin D status for cognitive functioning in humans is not known. A recent study reported an association between serum 25-(OH)D_3 levels and cognitive functioning in a relatively small cohort of patients referred to a memory clinic[12].

Because of these new insights, we examined the relationship between vitamin D status and cognitive function in a cohort of older patients with probable

AD in our memory clinic. We also analyzed the serum levels of vitamin B_1 , B_6 and B_{12} , as screening for deficiencies in these vitamins is recommended in the clinical guidelines for the assessment of dementia.

METHODS

Patients

We included 962 consecutive patients who were referred to the geriatric outpatient clinic of the Erasmus University Medical Center. The patients were referred for a variety of reasons and underwent a comprehensive geriatric assessment, including history and informant history, medication history, and physical and neurological examination[13]. They were asked to complete the Dutch version of the Mini-Mental State Examination (MMSE), with scores ranging from 0 points (poor cognitive functioning) to 30 points (good cognitive functioning) [14]. A consensus diagnosis of probable AD was obtained for 350 patients by a multidisciplinary memory clinic team including 2 geriatricians, a neurologist, a neuropsychologist and a psychiatrist[15]. Seventy-seven percent also underwent a neuropsychological examination. After exclusion of patients taking any vitamin suppletion at the time of investigation, serum vitamin B_1 , B_6, B_{12} and 25-(OH) D_3 levels were available for 225 out of 350 patients. The study was approved by the medical ethics committee of the Erasmus University Medical Center. Oral informed consent was obtained from the patients and their main caregiver.

Dementia diagnosis

The diagnosis of dementia was verified according to a standard protocol. Dementia was diagnosed with reference to the American Psychiatric Association's criteria (DSM-IV) [16]. The subdiagnosis of probable AD was based on the criteria of the NINCDS-ADRDA work group[17].

Possible cofactors

The serum 25-(OH)D_3 level strongly depends on sunlight exposure and therefore data were collected on action radius and mobility as indicators for exposure to sunlight. The action radius was classified as going outside, being housebound or lying in bed > 20h per day. For scoring mobility we used a standardized mo-

bility scale assessing independence in walking, standing, transfers in and out of bed/chair, and wheelchair use. The score for each of these items ranged from 0 points (complete independence) to 3 or 4 points (complete dependence), with a maximum score of 14 points[18]. The total years of education were scored because both cognitive test performance and serum 25-(OH)D_3 level are influenced by educational background[19].

Blood parameters

Blood samples were collected on the first visit to the outpatient clinic. The serum 25-(OH)D_3 levels were measured using a radioimmunoassay (DiaSorin). The serum vitamin B_1 and B_6 levels were determined by high-performance liquid chromatography according to internal protocols of the clinical laboratory of the Erasmus University Medical Center. The serum vitamin B_{12} levels were measured using a competitive protein-binding assay according to internal protocols of the clinical laboratory of the Erasmus University Medical Center. All assessments were performed at the Erasmus University Medical Center.

Statistical analysis

The association between serum vitamin levels [25-(OH)D_3, vitamin B_1, B_6 and B_{12}] and MMSE score (dependent variable) was investigated by linear regression. To adjust for possible confounders, the models were adjusted for biologically plausible cofactors. These cofactors were age, gender, total mobility score, action radius, years of education and the vitamin levels not under investigation. They were added to the model one by one as linear covariables. Furthermore, the mean MMSE scores were compared between vitamin-D-insufficient and vitamin-D-replete patients. The cutoff point for defining vitamin D deficiency was set at a serum 25-(OH)D_3 level < 50 nmol/l[20]. SPSS version 12.0 was used for statistical analysis (SPSS Inc., Chicago Ill., USA).

RESULTS

The characteristics of the population are shown in Table 1. The mean MMSE score was 19.7 ± 6.3 points and the mean serum 25-(OH)D_3 level was 45.4 ± 22.8 nmol/l. In this cohort 141 out of 225 individuals (63%) had a serum 25-(OH)D_3 level < 50 nmol/l and 197 out of 225 (88%) had serum 25-(OH)D 3 levels < 75 nmol/l.

Table1. Characteristics of study population (N = 225)

Characteristics	Mean ± SD	Range
Age (years)	77,6 ± 7,3	60 - 94
Gender: female, no. (%)	147 (65%)	-
MMSE score (points)	19,7 ± 6,3	1 - 30
Mobility score (points)	1,3 ± 2,8	0 - 14
Education (years)	8,8 ± 3,1	3 – 20
s-25(OHD_3, nmol/l (normal range > 50)	45,4 ± 22,8	5 - 106
s-vitaminB_1, nmol/l (normal range: 70-140)	105,1 ± 26,3	47 – 167
s-vitamin B_6, nmol/l (normal range: 46-126)	63,3 ± 44,5	26 – 151
s-vitamin B_{12}, pmol/l (normal range: 145-637)	304,5 ± 137,7	60 - 755

SD = standard deviation

The results of the regression analysis studying the association between serum 25-(OH)D_3 levels and MMSE scores are shown in Table 2. A positive association between serum 25-(OH)D_3 levels and MMSE score was observed with a β-coefficient of 0.08 ($p < 0.001$). This finding remained significant after adjustment for possible confounders ($\beta = 0.05$; $p = 0.01$). The mean MMSE score in vitamin D-deficient patients [25-(OH)D_3 < 50 nmol/l] was 18.5 ± 6.0 points and that in vitamin-D-replete patients was 21.5 ± 6.1 points ($p = 0.003$). Figure 1 shows a scatter plot of the MMSE score versus serum 25-(OH)D_3 level. Both in the adjusted and unadjusted models no significant associations were observed between serum vitamin B_1, B_6 and B_{12} levels and MMSE score (Table 2).

Table 2. Linear regression (β) coefficients describing the association between MMSE score (dependent variable) and serum vitamin levels

AD subgroup (N = 225)	Unadjusted β coefficient	P value	Adjusted β coefficient	P value
s-25(OH)D_3 level	0,08	< 0,001	0,05	0,01
s-vitamin B_1 level	0,03	0,1	0,02	0,3
s-vitamin B_6 level	0,003	0,8	0,005	0,6
s-vitamin B_{12} level	-0,001	0,9	-0,002	0,5

Adjusted: age, sex, mobility score, action radius, total years of education and s-vitamin levels not under investigation

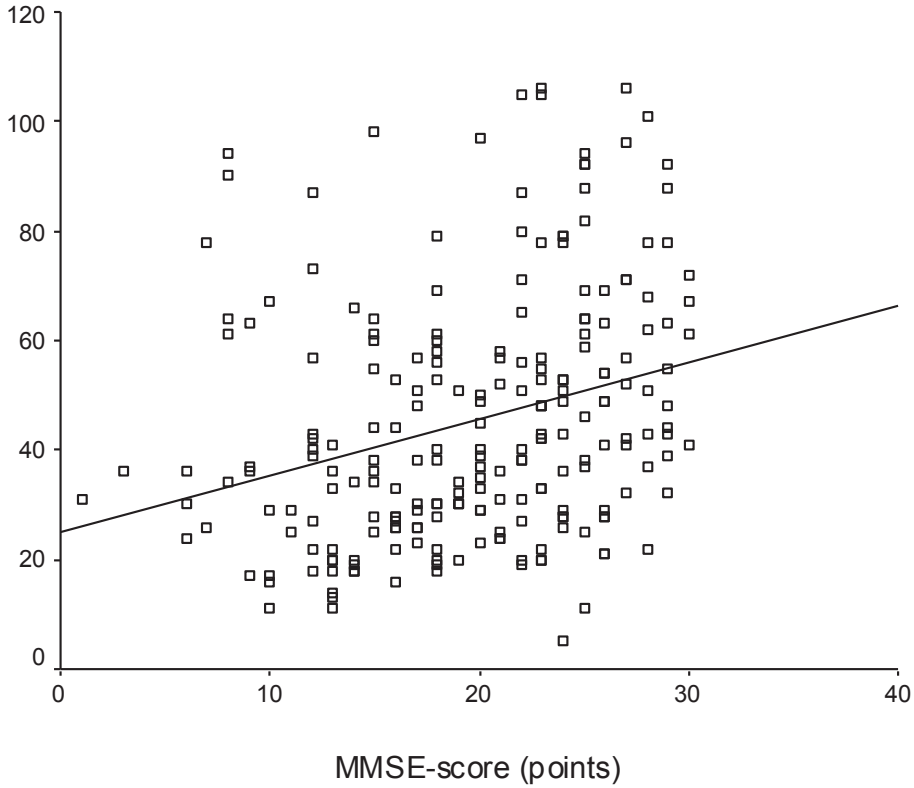

Figure 1 Scatter plot of the MMSE score (x-axis) versus s-25OHD_3 level (y-axis)

DISCUSSION

In the present study, performed in a geriatric outpatient population, we tested the hypothesis that the serum 25-(OH)D_3 levels are related to the level of cognitive functioning in patients with probable AD. We found an association between MMSE scores and serum 25-(OH)D_3 levels. No link was observed between MMSE scores and serum vitamin B_1, B_6 or B_{12} levels. This may be due to the fact that the mean serum vitamin B_1, B_6 and B_{12} levels were within the normal range.

The high prevalence of vitamin D deficiency in our patients is in accordance with other reports both from the Netherlands and abroad[21,22].

Our study is, to the best of our knowledge, the largest to date examining the association between serum 25-(OH)D_3 level and cognition in a cohort of older

AD patients. The results are in line with a previous study on the association between serum 25-(OH)D_3 levels and cognitive functioning[12].

Given the cross-sectional design of this study, the association between vitamin D status and MMSE scores can be interpreted in several ways. First, vitamin D deficiency may lead to a gradual decline in cognitive functions. Data, mostly from ex vivo and animal studies, suggest that vitamin D metabolites may have protective effects on neurons or neurotransmitter pathways which could benefit cognition. It has been proposed that vitamin D metabolites exert their protective effects on the central nervous system by stimulating the production of neurotrophins or by inhibiting the production of inducible nitric oxide[8]. A positive effect of 1,25-$(OH)_2D_3$ on the acetylcholine pathway has also been reported[23]. Recent studies suggest that 1,25-$(OH)_2D_3$ may also decrease the production of pro-inflammatory cytokines like tumor necrosis factor α. Tumor necrosis factor α is thought to play a pathogenic role in neurodegenerative disorders, such as AD and Parkinson's disease[24, 25].

Conversely, cognitive decline may cause vitamin D deficiency. It has been shown that AD can lead to malnutrition, which in turn could contribute to the development of vitamin D deficiency[26]. In addition, exposure to sunlight might decrease due to behavioral problems in AD, such as apathy and inertia, leading to more time being spent indoors[27].

A reduction in VDR expression in different layers of the hippocampus in patients with AD has been reported[28]. The hippocampus is the most typical part of the brain involved in AD. The regulators of VDR expression in the central nervous system are still largely unknown. In other cell types such as muscle cells, a decreased expression of the VDR, independent of serum vitamin D level, has been described with aging[29]. However, contradictory results, which question the role of vitamin D metabolites in the preservation of cognitive function, come from a recent animal study that showed muscular and motor impairments, but no impairments in cognition, in VDR knockout mice compared to wild-type mice[30].

Our study has some limitations. First, because of the cross-sectional design, no causality can be concluded. However, it seems worthwhile to gain more insight into the association between vitamin D deficiency and cognitive impairment, as supplementation of vitamin D might be an inexpensive and safe intervention[31].

Second, the MMSE is a crude measure of cognitive functions and fluctuations in cognition could have been missed[32]. However, AD diagnosis in our patients was verified by neuropsychological testing and based on a thorough multidisciplinary diagnostic process. In view of the high prevalence of vitamin D deficiency in our rather mobile outpatients, our study supports the recommendations for preventative vitamin D supplementation in older people at risk[22].

In conclusion, the results of the present study suggest that an association exists between vitamin D status and cognitive functioning in AD. Further prospective studies with a long follow-up period and with more elaborate data on cognitive function and cognitive test performance are needed to specify the possible contribution of vitamin D deficiency to the onset and course of cognitive decline and AD. In the meantime, we should continue to recognize that vitamin D deficiency is prevalent in older persons. Quite apart from the consideration that vitamin D metabolites may have specific neuroprotective effects, the need to identify and treat comorbid conditions in AD patients remains unchanged[33].

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Section 4

Vascular function

Chapter 4.1

Serum vitamin D levels are associated with structural and functional properties of the carotid artery in older men and women

Christian Oudshoorn, Edgar M. Colin,
Suzanne C. van Dijk, Astrid G. Ruitenbeek, Anton H. van den Meiracker,
Tischa J.M. van der Cammen and Francesco .U.S. Mattace-Raso

Submitted

ABSTRACT

Aim: In this study we investigated the association between serum vitamin D levels in relation to carotid and radial artery distensibility.

Methods: Older men and women referred to the outpatient clinic of the department of internal

medicine and geriatric medicine were asked to participate in this cross sectional study. Carotid and radial artery distensibility coefficients were measured. Linear regression analyses were performed to investigate the association between serum 25(OH)D levels and carotid and radial distensibility.

Results: The mean age of this population was 77.9 years and 28/49 (57%) were women. The mean serum 25(OH)D level was 50±28.8nmol/L. Serum 25(OH)D level was associated with carotid artery distensibility (β = 0.067; 95% CI, 0.021, 0.113). No association was observed with radial artery distensibility (β = 0.015; 95% CI, -0.031, 0.061). An association was also observed between serum 25(OH) D level and carotid artery intima-media thickness (β = -0.002; 95% CI, -0.004, 0.00).

Conclusion: In this cohort of older men and women serum 25(OH)D levels were associated with both structural and functional properties of the carotid artery. No association was observed with the radial artery distensibility.

INTRODUCTION

Vitamin D, a seco-steroid hormone, is mostly known because of its importance for calcium homeostasis and bone health. Vitamin D is obtained through cutaneous synthesis resulting from sun exposure, and through oral intake from food and supplement sources[1]. In recent years it has become clear that the vitamin D receptor (VDR) via which the vitamin D metabolites exert their function, is expressed by almost all cells in the human body[2]. Vitamin D deficiency, as assessed by circulating 25-hydroxyvitamin D_3 (25(OH)D) levels, has in recent years drawn attention as a potential risk factor for cardiovascular disease (CVD)[3]. Vitamin D deficiency is thought to influence CVD risk predominantly by acting on established CVD risk factors, such as hypertension, insulin resistance, and inflammation[4]. Recent publications suggest that vitamin D deficiency is also associated with and increased risk of aortic stiffness[5, 6].

Previously, we found that central and peripheral arteries can be affected differently in regards to structural and functional properties[7]. In addition, if vascular stiffening (i.e. a decrease in vascular distensibility) is associated with vitamin D status and if there is a difference between various vascular regions is still unclear. The aim of this study is to assess whether mean serum 25(OH)D levels are associated with structural and functional arterial properties in a cohort of older persons.

METHODS

Study Population

Consecutive patients referred to the outpatient clinic of the Department of Internal Medicine and Geriatric Medicine, of the Erasmus Medical Center, were asked to participate in the study. Patients with a cardiovascular event within 6 weeks before the visit were excluded from the study. Patients aged 55 years and older were included. Informed consent was obtained from all patients.

Cardiovascular Risk Factors

Information on previous cardiovascular disease, smoking habits, and drug use was obtained by interview. Patients were classified as ever-smokers (current or past smokers) or never-smokers. Patients' height and weight were measured,

and body mass index (BMI; weight [kg]/height^2[m]) was calculated. Diabetes mellitus was defined as the use of blood glucose–lowering medication or a fasting serum glucose level equal to or greater than 7.0 mmol/L.

Blood Pressure Measurements

Blood pressure and heart rate were measured twice on the right arm using an automatic device (Accutorr Plus; Datascope Corporation, Mahwah, New Jersey). The measurements were obtained after at least 5 minutes of rest with the patient in the supine position. The pulse pressure (PP) was calculated as systolic blood pressure (SBP) - diastolic blood pressure (DBP). The average of the 2 measurements was used in the analysis.

Arterial Distensibility Measurements

After at least 5 minutes of rest with the patient in the supine position, arterial measurements were performed by Wall Track System 2 (Pie Medical, Maastricht, the Netherlands), using a B-mode ultrasound to identify the right common carotid artery at 1 to 2 cm proximal to the origin of the bulb. The right radial artery was investigated at the antecubital crease. The end-diastolic diameter (D), the absolute stroke change in diameter during systole (ΔD), and the relative stroke change in diameter (ΔD/D) were computed as the mean of values measured in 4 seconds of 3 successive recordings. The distensibility coefficient (DC) was calculated by the following equation: 2(ΔD/D)/PP (10 MPa−1). The means of diameter and distension of 3 successive recordings were taken as the subject's readings.

Carotid artery intima-media thickness measurement

The maximum common carotid intima-media thickness (IMT) was determined as the average of the maximum IMT of near- and far-wall measurements over a length of 1 cm, and the average of left and right maximum common carotid IMT was computed.

Measurement serum vitamin D levels

Blood samples were collected the same day as the cardiovascular measurements were performed. The measurements were taken during a short period of time, from April until July. Serum 25(OH)D levels were measured using a radioimmunoassay (DiaSorin).

Statistics

Mean values with standard deviation and percentages were calculated for continuous and categorical variables respectively. The association between serum 25(OH)D levels and the various cardiovascular parameters (dependent variable) was investigated by linear regression. Models were adjusted for possible confounders (age, gender, MAP, use of anti-hypertensives and heart rate). All analyses were performed using Statistical Package for the Social Sciences (SPSS) software (version 21.0). A p-value <0.05 was considered statistically significant.

RESULTS

The characteristics of the study population are shown in Table 1. The mean systolic blood pressure was 130±15.5mmHg, and the mean diastolic blood pressure was 71 ± 9.1mmHg. The mean serum 25(OH)D level was 50 ± 28.8nmol/L. In this cohort 28 out of 49 participants (57%) had serum 25(OH)D levels ≤ 50nmol/L and 39 out of 49 (80%) had serum 25(OH)D levels ≤ 75nmol/L.

Table 1 Baseline characteristics of the study participants

Characteristic	Mean ± SD (N= 49)
Age, years	77,9 ± 7,8
Female, n (%)	28 (57%)
Smokers, %	9 (18%)
Anti-hypertensive therapy, %	20 (41%)
Diabetes mellitus, %	3 (6%)
Vitamin D supplement use, %	8/49 (16%)
Serum 25(OH)D (nmol/L)	50,3 ± 28,8
BMI, Kg/m^2	25,6 ± 5,4
SBP, mmHg	130,8 ± 15,5
DBP, mmHg	71,1 ± 9,0
PP, mmHg	59,7 ± 11,4
MAP, mmHg	94 ± 11,8
IMT, mm	1,1 ± 0,6
Carotid distensibility, 10−3/kPa	11,0 ± 5,2
Radial distensibility, 10−3/kPa	7,5 ± 4,1

BMI = body mass index; SBP = systolic blood pressure; DBP = diastolic blood pressure, PP = pulse pressure; IMT = carotid artery intima-media thickness; MAP = mean arterial pressure

The results of the regression analysis studying the association between serum 25(OH)D levels and carotid and radial distensibility are shown in Table 2 and Fig-

ure 1. After adjustment for possible confounders a significant association was observed between serum 25(OH)D levels and carotid distensibility (β = 0.067; 95% CI, 0.021, 0.113). No association between serum 25(OH)D level and radial distensibility was observed (β = 0.015; 95% CI, -0.031, 0.061). Furthermore, a positive association was observed between serum 25(OH)D level and carotid artery IMT (β = -0.002; 95% CI, -0.004, 0.00). No association observed between serum 25(OH)D levels and either pulse pressure, SBP or DBP (Table 2).

Table 2 Linear regression β-coefficients and 95% Confidence Interval (95% CI) describing the association between serum 25(OH)D levels and various vascular properties

Characteristic	β coefficient[a]	95% CI
Carotid distensibility	0.067	0.021, 0.113
Radial distensibility	0.015	-0.031, 0.061
Systolic blood pressure	-0.026	-0.090, 0.037
Diastolic blood pressure	0.034	-0.009, 0.077
Pulse pressure	-0.060	-0.157, 0.037
Intima-media thickness	-0.002	-0.004, 0.000

[a]Model adjusted for age, gender, Mean arterial Pressure, anti-hypertensive therapy and heart rate

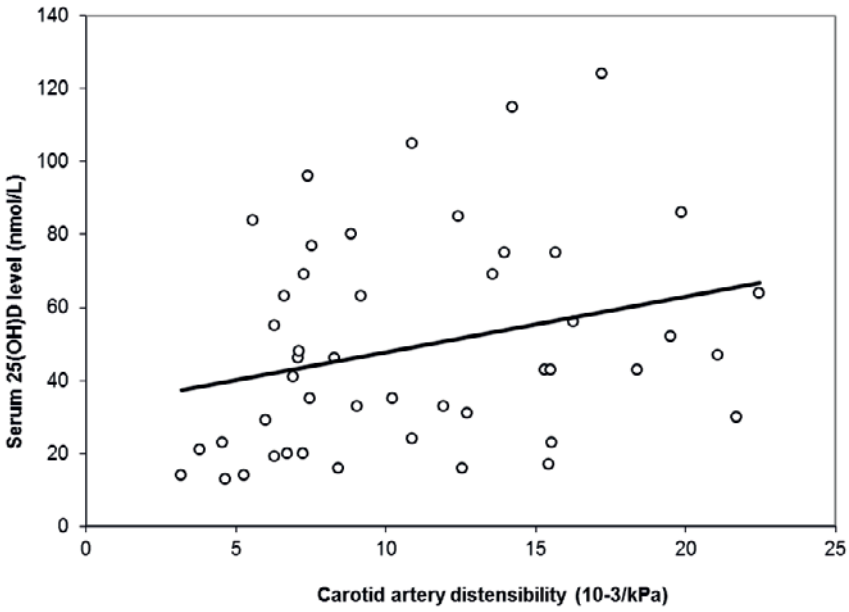

Figure 1 A, Scatter plot of the association between serum 25(OH)D level and carotid artery distensibility coefficient, (P = 0.005).

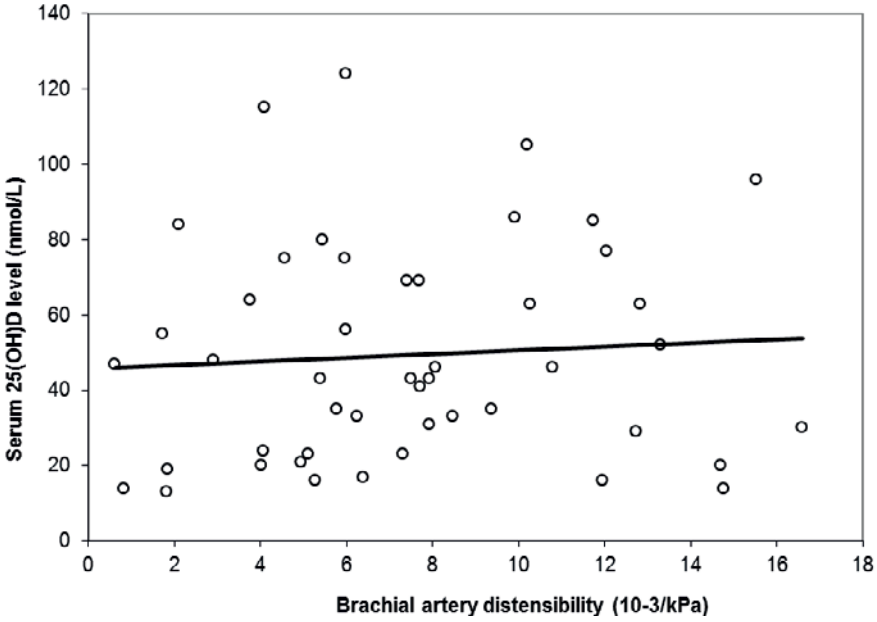

Figure 1 B, Scatter plot of the association between serum 25(OH)D level and radial artery distensibility (P = 0.51)

DISCUSSION

In the present study performed in older men and women referred to the outpatient clinic, serum 25(OH)D levels were associated with both carotid distensibility and carotid artery intima-media thickness. No association of vitamin D status with radial distensibility was observed. The results from this cross-sectional study suggest that serum vitamin D levels could affect vascular structural and functional properties and the results also suggest that various vascular territories could be affected differently.

There is accumulating evidence that vitamin D deficiency is associated an increased risk for cardiovascular disease. Large population based studies have previously shown an association between poor vitamin D status and an increased risk of cardiovascular events and cardiovascular related mortality, and have also suggested that vitamin D could be important in the prevention of vascular calcification[8]. Arterial stiffness increases with age and is a predictor of morbidity and mortality[9]. Well established risk factors for arterial stiffening

are hypertension, diabetes mellitus and inflammation[10]. However, a role for vitamin D in the process of arterial stiffening has also been suggested[8]. Several studies reported on the association between serum 25(OH)D levels and arterial stiffness. In the Baltimore Longitudinal Study of Aging, a prospective study of normative aging, vitamin D status was inversely associated with carotid-femoral pulse wave velocity (PWV) in a cohort of 1228 healthy volunteers with a mean age of 70 years[6]. A recent cross sectional study by Mayer et al. in a relatively young population (mean age 52.8 years) reported an association between vitamin D status and aortic pulse wave velocity[5].

The exact role of vitamin D in cardiovascular health and specifically the role in arterial stiffening is thought to be multifactorial. Both animal and human studies have shown that vitamin D metabolites are a negative regulator of the renin-angiotensin-aldosterone system (RAAS)[11]. The RAAS is involved in maintenance of blood pressure, electrolyte homeostasis and control of intravascular volume. Large prospective studies have shown an association between low vitamin D levels and increased activity of RAAS which may result in hypertension and increased water intake and sodium absorption[11]. Furthermore, vitamin D metabolites are thought to directly alter myocyte contractility and proliferation[12]. Effects of vitamin D have also been reported on endothelial function, regulation of vascular endothelial growth factor (VEGF) production and on insulin and glucose handling[4].

It has previously been reported that vascular stiffening can differ between various vascular regions. In a study by Ruitenbeek et al. we found that age and blood pressure influenced functional properties of the elastic central arteries but not the muscular peripheral arteries[7]. Based on the results of the present study, it can also be hypothesized that central and peripheral arteries are affected differently by vitamin D deficiency in regard to their functional and structural properties as associations are observed with carotid distensibility and IMT, while no association is observed with radial distensibility.

Our study has several limitation. First, given the relatively small number of participants generalizability is limited. Second, the observational, cross-sectional design of the study precludes definitive conclusions regarding causality. Third, no information was available on the use of lipid lowers medication.

In conclusion, in a group of older men and women from the outpatient clinic, serum 25(OH)D level was associated with both structural and functional properties of the carotid artery. No association was observed with the radial artery distensibility.

DISCLOSURE STATEMENT

The authors declare no conflict of interest.

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Section 5

General discussion

GENERAL DISCUSSION

The main objective of this thesis was to gain more knowledge about the importance of vitamin D for older individuals in regard to functionality, with an emphasis on muscle, cardiovascular and neurological function. Vitamin D research has a long history. In the year 1922 the Dutch orthopedic surgeon Havinga published an extensive review on rickets in the Dutch language[1]. The author aimed to summarize the current knowledge on rickets whilst the literature in those days is already getting "too extensive for a person to comprehend". In the 1920's it was already established that the risk of developing rickets is related to latitude and a link with exposure to sunlight was suggested. Even then, certain effects outside bone tissue were already documented in children with rickets, such as specific muscle abnormalities. Treatment options in those days consisted mainly of salt baths and hot towel compression therapy, but the positive effects of exposure to sunlight or a quartz lamp were started to be recognized. The author concluded with the sentence: *"Dove non viene il sole, vien'il medico"*; which can be translated as: "*where the sun doesn't shine, the doctor appears*". Not long after the publication of this review, the hormone vitamin D is discovered by the American biochemist Elmer V. McCollum. In the forthcoming decades vitamin D research would develop gradually, and the importance of vitamin D for various disease states in old age would be increasingly recognized.

The main findings of this thesis are:

- Ageing increases the risk of vitamin D deficiency and is associated with changes in the calcium homeostasis which impairs the efficacy of the calcium homeostatic process
- The prevalence of vitamin D deficiency among older persons is still high despite strong attention for this hormone in recent years
- Public knowledge on vitamin D is poor and the level of knowledge is related to their vitamin D status
- There is a strong increase in incidence rates of both vertebral fractures and hip fractures in the oldest-old (>80 years) in recent years

- Vitamin D status in observational studies is related to various functional outcomes in older people such as muscle strength, mobility, cognitive performance and specific cardiovascular measures
- High dose vitamin D supplementation is effective in raising serum $25OHD_3$ levels in older persons and this dose is well tolerated
- High dose vitamin D supplementation in vitamin D deficient older persons improves gait speed

The main findings mentioned above are discussed in more detail in this chapter. Furthermore, methodological considerations concerning the main study outcomes are discussed. Finally, recommendations for future research are presented.

MAIN FINDINGS

The effects of ageing on calcium homeostasis and the vitamin D endocrine system

In the review in chapter 2.1 we summarized the effects of ageing on both the vitamin D endocrine system and calcium homeostasis. Ageing has considerable effects on both processes, which impairs their efficacy, leading to specific vulnerabilities in older people. Essential is the increased prevalence of vitamin D deficiency in old age. This is caused by both a decreased cutaneous production of vitamin D due to age related changes in the skin and life-style alterations such as less time being spent outdoors, and specific dietary changes. Other age-related changes are an altered metabolism of the various vitamin D metabolites and altered target tissue responsiveness to vitamin D[(2)].

In older individuals there is also a decrease in intestinal and renal calcium (re)absorption. In part, this decrease in efficiency of the calcium homeostatic process observed in elderly is vitamin D related. For example, the expression of intestinal calcium channels, necessary for intestinal calcium absorption, is up regulated by vitamin D. So, a state of vitamin D deficiency leads to a decrease in intestinal calcium channel expression and thus impairs calcium absorption leading to a negative calcium balance. However, the decreased intestinal calcium channel expression in older persons cannot entirely be explained by the occurrence of vitamin D deficiency. Some studies suggest that sex hormones

also stimulate intestinal calcium channel expression and the decrease in serum levels of sex hormones in older persons could contribute to the decrease in calcium channel expression[3].

In conclusion, the effects of ageing on the vitamin D endocrine system and calcium homeostatic process are complex. The effects impair the efficiency of both processes leading to specific vulnerabilities in older people either directly because of the vitamin D deficiency or because of the negative calcium (and phosphate) balance. The health consequences are diverse given the pleiotropic effects of the vitamin D hormone.

Prevalence of vitamin D deficiency and the interrelation with health knowledge

In our study, a cross sectional study among older individuals living in residential care facilities, 67% (285/426) of the participants were vitamin D deficient with a serum $25OHD_3$ level < 50nmol/l. Of those, 27% (115/426) had a serum $25OHD_3$ level < 25nmol/l. The results of this study demonstrate that despite the implementation of guidelines and the increasing attention for vitamin D in both research and media during the past decade, the prevalence of vitamin D deficiency among older persons in the Netherlands remains high. In a search for causes for this persisting high prevalence of vitamin D deficiency, we studied if health knowledge, and more specifically knowledge on vitamin D, was related to vitamin D status. In a cohort of older people living in residential care homes, we observed an association between knowledge (awareness) on vitamin D and serum $25OHD_3$ concentration. Patients with the best knowledge on vitamin D had higher serum $25OHD_3$ levels as compared to individuals who had the least knowledge. This study also demonstrated that overall, general knowledge on vitamin D among older persons is poor. Only 38% of the participants indicated that they knew or had heard of vitamin D. Among those that knew about vitamin D, there were many misconceptions or false believes regarding sources and effects of vitamin D. To date, mostly studies that examine knowledge on vitamin D in younger age groups have been published. In these studies awareness of vitamin D ranged from 70% to 97%[4-6]. The cause for this observed difference between older and younger individuals on awareness of vitamin D is not known but limited access to information (newspapers, internet), sensory impairment

(poor vision and hearing) and cognitive impairment could play a role. Interestingly, these studies also reported many misbelieves regarding sources and effects of vitamin D in young individuals.

Given the fact that there seems to be a general lack of knowledge on vitamin D, improving public knowledge might be an intervention strategy in the prevention of vitamin D deficiency. However, the possible association between knowledge on vitamin D and vitamin D status in older persons is still subject of debate. Both knowledge and attitude towards vitamin D are considered as prerequisites to specific health behavior. The interrelation between these variables seems complex. A previous study reported no association between knowledge and five vitamin D related behaviours (all actions people can take to improve vitamin D status)[7]. Furthermore, a Chinese study found that individuals with the best awareness on the possible benefits of sunlight and the importance of vitamin D for human health tended to avoid sun exposure[6]. In addition, a study in the USA found no effect of an increased knowledge of vitamin D on the dietary intake of vitamin D[8].

Time trends of the most common fractures among older persons

In this thesis we studied secular trends on the most common fractures in older individuals: vertebral fractures and hip fractures. These type of fractures are strongly associated with osteoporosis, for which vitamin D deficiency is an important risk factor. Trend analyses of the number of patients with a hip fracture over the last decades demonstrated a strong increase in fracture numbers until the mid-nineties. Since then, the increase in incidence rates of hip fractures started to slow down. Over the last decade, the overall incidence rate has even started to decrease. This trend has also been observed in several other countries[9, 10]. Interestingly, in our study there was a difference observed between the various age groups. While incidence rates in the younger age groups (<80 years) decreased, the incidence rates in the oldest old (> 80 years) still increased. A cause for this difference in trends that seems age related is not known. For the number of vertebral fractures seen at the emergency room over the last decades we observed a strong increase in all age groups. However, also with this fracture

type there was an age related trend observed: the strongest increase was seen in the oldest individuals (>80 years).

Vertebral and hip fractures are the most common fractures among older person[11]. Especially a hip fracture is typically associated with ageing, with approximately 85% of all hip fractures occurring in individuals aged ≥65 years[12]. Hip fractures are, more than any type of fracture, associated with loss of independence, morbidity and mortality[13]. Vertebral fractures are, just like hip fractures, associated with functional impairment and mortality. Over 90% of the vertebral fractures are associated with osteoporosis. A previous vertebral fractures is a strong predictor for a new vertebral fracture, but also for a hip fracture[14]. So, fracture prevention, including timely treatment of the underlying risk factors of both fracture risk and fall risk is of the utmost importance.

In recent years many care plan and protocols regarding fracture prevention have been implemented[15]. While this seems successful in the reduction of incidence rates of fractures, especially hip fractures in relatively young people, the effects in the oldest old seems to fall behind. The cause of these trends is not known, but they demonstrate a need for more focus on the optimalization of bone health in these older individuals. For the optimalization of bone health and the prevention of diseases like osteoporosis, the timely diagnosis and subsequent of vitamin D deficiency plays a crucial role, as this is a well-known risk factor for osteoporosis. Furthermore, fall risk should also be assessed in patients with a fracture after a fall. In the fall risk assessment, the diagnosis and treatment of vitamin D deficiency is also included[16]. So, improving vitamin D status in older individuals could potentially be a cheap and safe intervention to reduce fracture rates in the oldest old as still many patients, most likely, remain untreated.

Vitamin D and functional performance, with an emphasis on muscle, neurological and cardiovascular function

Muscle function

Even in healthy individuals there is considerable loss of skeletal muscle mass and strength (sarcopenia) with ageing. This process is further aggravated by an unhealthy (sedentary) life style and by diseases[17]. Commonly with muscle

ageing, the terminology of extrinsic or intrinsic age effects is used. These refer to extrinsic (e.g. hormonal changes and inflammatory effects) and intrinsic (e.g. telomere shortening or mitochondrial DNA damage) effects to the muscle cells or satellite cells[18]. Since the discovery of VDR expression in skeletal muscle tissue, hypovitaminosis D is regarded as an important extrinsic factor that contributes to the process of sarcopenia with ageing.

In recent years, many studies have been performed on vitamin D and muscle function. Evidence from observational studies is generally consistent and shows an association between vitamin D status and various measures of physical performance such as quadriceps strength, handgrip strength, balance and gait speed[19]. Generally, some signs and symptoms of musculo-skeletal dysfunction such as falls are especially prevalent among older women suggesting that muscle performance in men and women might be affected differently by states of vitamin D deficiency. Furthermore, some recent observational studies failed to find an association between functional performance in men and vitamin D status, questioning the importance of vitamin D for muscle performance in men[20]. However, our study demonstrated that functional performance is associated with vitamin D status in both men and women suggesting that functional performance in both men and women is equally affected by vitamin D status. This is an important insight in order to prevent under-diagnosis and –treatment of vitamin D deficiency in men. In a closely related disease, namely male osteoporosis, this has happened until fairly recent, resulting in an underestimation of the fracture risk in men.

While the results of observational studies on vitamin D and muscle function, especially in women, seem fairly consistent, the result of intervention studies are far less consistent. A recent extensive umbrella review of 87 meta-analyses of randomized controlled trials that studied the effect of vitamin D supplementation on 137 different clinical endpoints concluded that there was considerable doubt regarding a possible role of vitamin D in almost all clinical endpoints[21]. The authors of this review concluded that the argument that vitamin D supplementation reduced the risk of falls or improved muscle performance measures in older people was doubtful. The evidence regarding a possible effect on muscle strength was classified as “suggestive”. This contrasts with previous reports that suggested a more clear effect of vitamin D on muscle strength,

physical performance, gait speed and balance[22, 23]. The proposed causes for the inconsistencies observed between various vitamin D intervention trials are variation in study population, number of participants (lack of sufficient power), variation in baseline vitamin D status and functional performance, variation in intervention duration and variation in supplementation dose. Especially the effective supplementation dose (or optimal serum $25OHD_3$ level for muscle function) has been subject of debate. Recent studies suggested that a serum $25OHD_3$ level of 75- 90nmol/l is preferable for optimal muscle function[24, 25]. This serum concentration, however, is not reached in most trials. In our study, a high supplementation dose was used of 1800IU/day, for six months. This resulted in a mean serum $25OHD_3$ level of 107 nmol/l. This supplementation dose was well tolerated. In this study an improvement in gait speed and timed up go (TUG) performance was observed, while no effect on muscle strength was observed. The observed results contrast some previous reports but are in line with other intervention trials[26, 27]. In our study gait speed improved with about 8%, while in absolute measures, the mean improvement was 0.08m/s. These results demonstrate that there is at least some effect of vitamin D supplementation on functional performance. However, in the literature, a clinical relevant change in gait speed is regarded to be at least an improvement of 0.1m/s making the clinical relevance of the observed effect questionable. Furthermore, previous studies also suggested an improvement of muscle strength and performance with vitamin D supplementation of about 8%. These trials, however, used a relatively low dose vitamin D supplementation of mostly 400IU- 800IU/day. Therefore, the observed effect in our study make the additional effect and necessity of high dose supplementation less clear as the functional improvement in our study was not superior to the improvement observed in low(er) dose supplementation trials[26].

Cardiovascular function

The hypothesis that vitamin D status is associated with cardiovascular health seems plausible given the wide distribution of the VDR among tissues involved in blood pressure regulation[28]. However, importance of vitamin D for cardiovascular health is still not clear. Possible roles for vitamin D are still being unraveled. In our study we found an association between vitamin D status and

carotid intima media thickness (IMT) while no association was observed with radial IMT. Based on the results of this study it can be hypothesized that various blood vessels, either central or peripheral, are affected differently by vitamin D. Correct understanding of the significance of vitamin D for cardiovascular health is important to eventually understand both the risk of both vitamin D deficiency and also vitamin D supplementation for cardiovascular health.

In recent years various studies have been published that further complicate the possible role of vitamin D in cardiovascular health. In studies published by Durup et al. and Wang et al., a non-linear relationship between serum $25OHD_3$ and cardiovascular disease was reported[29, 30]. Additionally, a recent study from the Netherlands by van Dijk et al. reported a non-linear association between serum $25OHD_3$ and carotid IMT and pulse wave velocity (PWV)[31]. In particular, above levels of 50nmol/l there was a slight increase of IMT with increasing $25OHD_3$ levels. This seems in line with the studies by Durup et al. and Wang et al. who reported a nadir around 60nmol/l in the risk curve between vitamin D status and cardiovascular disease and mortality. This uncertainty, concerning the possible non-linear association between vitamin D and cardiovascular disease, warrants some restraints in the use of high dose vitamin D supplementation. Especially given the fact that the additional benefit of high dose supplementation is still not proven and that the clinical experience with prolonged high dose vitamin D supplementation is still limited, both in young and old individuals.

Neurological function

In this thesis we demonstrated an association between vitamin D status and cognitive functioning in patients with symptoms of cognitive dysfunction. Since the study by Eyles et al. on the wide distribution of the VDR expression and the detection of the various vitamin D metabolites, the central nervous system is regarded a target tissue for vitamin D[32]. In animal models there seems clear evidence for the involvement of vitamin D in the normal neurological and brain development[33]. A recent study suggested that the threshold of $25OHD_3$ associated with cognitive impairment is around 25nmol/l (25nmol/l), persons with $25OHD_3$ concentrations <25nmol/l having a greater risk of cognitive disorders than those with $25OHD_3$ levels >10ng/ml[34].

However, to date, most clinical evidence on the importance of vitamin D for neurological function and more precise, cognitive function, comes from observational (cross-sectional) data. In our study there was a clear association between cognitive function and vitamin D status. It can therefore be hypothesized that vitamin D status is important for cognitive functioning, even in patients with memory complaints. In in vitro and ex vivo studies effects of vitamin D have been reported on neurotrophin levels, neurotransmitter production and free radical (nitric oxide) production which could affect cognitive function, strengthening the hypothesis that vitamin D is important for normal neurological functioning[35]. It should be noted, however, that given the cross-sectional nature of our study, no causality could be concluded. In these cross-sectional studies, there is always the possibility of reverse causality (see limitation section).

METHODOLOGICAL AND THEORETICAL ISSUES

Observational vitamin D data

Observational studies, no matter how well prepared or controlled, are still potentially subject to some methodological issues. This is a particular problem for observational data from vitamin D related studies. Firstly, almost all disease states are likely to reduce and persons' level of physical activity and therefore altering exposure to sunlight. This introduces the possibility of reverse causality in these studies. This issue affects all cross-sectional and longitudinal studies with short follow-up times. Secondly, several risk factors for disease states such as sarcopenia, dementia and cardiovascular disease are also known to lead to vitamin D deficiency. These risk factor include ageing, unhealthy diet and smoking. Low serum $25OHD_3$ levels could therefore possibly be a "bystander" (confounder).

Use of administrative databases

High quality electronic databases (the National Hospital Discharge Registry and the Dutch Injury Surveillance System) used for the fracture trend analyses in this thesis enable studies for trend analysis on specific topics over a long period. There are some limitations however. First of all, the data are only accurate within

the limitations of the coding system. The accuracy of the hospital information is likely to be dependent on the accuracy of the data in the medical records and the recognition of the injuries by the patient's physician and secondly by the clerk coding the diagnosis into the database. A second limitation of the administrative databases is that the databases do not contain information regarding factors such as underlying co-morbidities, drug use and clinical details of the individual patients[36].

Implications for clinical practice and future research

The studies in this thesis demonstrate that older persons are at risk for vitamin D deficiency and various other age related effects that increase fall and fracture risk. We demonstrated that especially in the oldest old (age ≥ 80 years) incidence rates of hip and vertebral fractures are increasing sharply in recent years. We also demonstrated that the prevalence of vitamin D deficiency is still high in these age groups. The high prevalence of vitamin D deficiency seems to persist despite the fact that the deleterious effects of vitamin D deficiency are well known and also despite the introduction of many guidelines, protocols and care plans on the screening and treatment of vitamin D deficiency. Given the observed trend in fracture rates, healthcare providers should focus more on this age group with regard to prevention of falls and fractures. The diagnosis and treatment of vitamin D deficiency in older individuals in this regard is essential. Future research should focus on factors related to the persisting high prevalence of vitamin D deficiency. In this regard, we demonstrated that there is a clear lack of knowledge among older individuals on vitamin D. It can be hypothesized that providing information on vitamin D to older people eventually leads to an improvement of vitamin D status and reduction in fracture rate. This hypothesis should be tested in future studies to develop an effective method to inform older persons. An effective method to inform the older population is not known at the moment and is most likely subject to change, given the recent technological advances.

In this thesis we also focused on the association between functionality and vitamin D in older individuals and more specifically the association with muscle function, cognitive function and cardiovascular function. In the section on vitamin D and muscle function we demonstrated that there is an association

between vitamin D status and muscle strength and mobility in both in men and women. Previous intervention studies on the effect of vitamin D supplementation show conflicting results with regard to muscle function. While this, in part could be due to methodological issues such as trial duration or participant selection, it has also been hypothesized that the supplementation dose in previous trials was too low. In a high dose intervention we saw improvement in gait speed and TUGT performance, while there was no effect on muscle strength. However, the clinical relevance of the observed effect was doubtful and did not seem superior when compared to normal or low dose vitamin D supplementation. So, while the trial results suggest there is at least some reversibility of the prolonged effects of hypovitaminosis D on muscle function with vitamin D supplementation, new trials on the effect of vitamin D are needed. Especially trails with a long observation period are urgently needed. These trials should also deal with other methodological issues of previous studies such as population heterogeneity, study power, variation in baseline vitamin D status and functional performance and supplementation dose.

In addition, we also demonstrated associations between vitamin D status and cardiovascular health and cognitive functioning. The observed associations strengthen the hypothesis that vitamin D could be associated with various disease states outside the musculo-skeletal system. So, future trials should ideally have a holistic approach on the possible effects of vitamin D and define a serum vitamin D level that is optimal for all target tissues. For example, the optimal serum vitamin D level or supplementation dose for muscle function is not necessarily the same as for cardiovascular health or neurological functioning. Future trials, especially those that use high dose supplementation should therefore include multiple endpoints of various organ systems and use a more holistic approach.

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Section 6

Summary and conclusions

Chapter 6.1

Summary and conclusions

Functional performance declines with ageing. During the ageing process all tissues and organ systems decline in function, resulting in a decreased functional reserve and a state of increased vulnerability. In this regard, vitamin D seems of particular interest because of the pleiotropic function of this hormone which include effects on muscle, bone, neurons and vascular tissue. Additionally specific age-related changes occur with regard to vitamin D and calcium homeostasis leading to specific risks and deficiencies in older individuals which contribute to the occurrence of functional decline in older individuals.

Section 1 gives a general introduction on the research topics of this thesis. Section 2, Chapter 2.1 provides a review of the specific age related changes that occur in the vitamin D endocrine system and calcium homeostatic process. Essential is the increased prevalence of vitamin D deficiency in older individuals. This is caused by both a decreased cutaneous production of vitamin D due to age related changes in the skin and life-style alterations such as less time being spent outdoors and specific dietary changes. In Chapter 2.2 the prevalence of vitamin D deficiency and the interrelation with health knowledge is presented. In older individuals living in residential care 67% (285/426) had a vitamin D deficiency (serum $25OHD_3$ level of <50nmol/l). There was a clear association between vitamin D related health knowledge and vitamin D serum level: participants who indicated that they knew vitamin D had a higher serum vitamin D level than those who indicated never to have heard of vitamin D. In total, only 38% of the participants indicated that they knew or had heard of vitamin D. Among those that indicated that they knew vitamin D, there were many misconceptions and false believes regarding the sources and effects of vitamin D. In Chapter 2.3 and 2.4 we present secular trends on the most common fractures in older individuals: vertebral fractures and hip fractures. These fractures are strongly associated with osteoporosis, for which vitamin D deficiency is an important risk factor. In Chapter 2.3 the number of hip fractures in the Netherlands is presented. The incidence of hip fractures increased rapidly until the mid-nineties. Since then, the increase in incidence rates of hip fractures started to slow down. Over the last decade, the overall incidence rate has started to decrease. There was a difference observed between the various age groups however. While incidence rates in the younger age groups (<80 years) decreased, the incidence rates in the oldest old (> 80 years) still increased. The cause of this trend is not known. In Chapter 2.4

the number of acute vertebral fractures leading to an Emergency Department visit in the Netherlands are shown. Overall, the number of vertebral fractures increased strongly over the past decades in almost all age groups. However, also with vertebral fractures, the strongest increase was seen in the oldest old. Overall, these observations demonstrate the need for more emphasis on falls and fracture prevention in the oldest old.

In Section 3, the interrelation between vitamin D and functional performance in older individuals is studied, with a focus on muscle, neurological and cardiovascular function. Firstly, in Chapter 3.1 the association between vitamin D status and physical performance in older individuals is presented. In our study, an association between functional performance and vitamin D status was observed in both men and women. This study negates the hypothesis that there is no association between vitamin D status and functional performance in men. In Chapter 3.2 the effect of high dose vitamin D supplementation on neuromuscular function is shown. High dose supplementation (1800IU/day) for six months was well tolerated and was effective in raising serum $25OHD_3$ levels. The intervention lead to an improvement in gait speed and timed up and go performance. No improvement in muscle strength was observed. The observed effect however was relatively small and not greater than in previous low dose intervention studies. In Chapter 3.3 the association between vitamin D status and cognitive performance is presented. In this chapter we demonstrated an association between cognitive performance and vitamin D status in patients with cognitive impairment.

In Section 4 the association between vitamin D status and vascular function is studied. The finding in this study indicates that various vascular regions in the body could be affected differently by states of vitamin D deficiency, demonstrating that the association between vascular health and vitamin D status is complex. Correct understanding of the interrelation between vitamin D status and vascular function is important because some studies previously reported an U-shaped association between vitamin D status and vascular health, where both very low and very high serum levels lead to an increased risk for vascular disease.

In Section 5, the general discussion, the main findings of this thesis are discussed and put in a broader perspective. The results indicate that vitamin

D deficiency is still prevalent among older individuals and that knowledge among older individuals about vitamin D is poor. With regard to functional performance we demonstrated an association between vitamin D status and muscle performance in both men and women. High dose vitamin D supplementation improves neuromuscular function (gait speed and TUGT performance) and is well tolerated. In addition associations were also observed with cognitive performance and vascular function. Future research should focus on defining the optimal serum vitamin D level or supplementation dose.

Chapter 6.2

Samenvatting en conclusies

Tijdens het verouderingsproces treedt er functieverlies op. Veroudering resulteert in achteruitgang van de verschillende orgaanfuncties waardoor er minder functionele reserve is en de kwetsbaarheid van een individu toeneemt. Hierbij speelt ook het hormoon vitamine D een belangrijke rol, vanwege de pleiotrope functies van dit hormoon, dat effecten heeft op onder andere spieren, botten, neuronen en bloedvaten. Het vitamine D-endocriene systeem en de calcium homeostase zijn aan bepaalde leeftijdsgerelateerde verouderingseffecten onderhevig die kunnen bijdragen aan het optreden van functieverlies tijdens het verouderingsproces.

Sectie 1 geeft een algemene introductie op de onderwerpen van dit proefschrift. Sectie 2, Hoofdstuk 1 beschrijft een review over de specifieke leeftijdsgerelateerde veranderingen die optreden binnen het vitamine D-endoriene systeem en de calcium homeostase. Essentieel hierin is de hoge prevalentie van vitamine D deficiëntie onder ouderen. Dit ontstaat met name door een verminderde productie van vitamine D in de huid door leeftijdsgerelateerde veranderingen in de huid, en door veranderingen in levensstijl waaronder het minder buiten zijn, en veranderingen in het dieet. In Hoofdstuk 2.2 wordt de prevalentie van vitamine D deficiëntie beschreven, evenals de samenhang tussen vitamine D deficiëntie en de specifieke kennis van ouderen over vitamine D. Bij onderzoek onder oudere personen wonend in een verzorgingshuis, bleek bij 67% (285/426 personen) sprake van een vitamine D deficiëntie (serum $25OHD_3$ concentratie < 50nmol/l). Er was een duidelijke associatie tussen de kennis die een oudere had omtrent vitamine D en de serum $25OHD_3$ concentratie: personen die aangaven vitamine D te kennen hadden een hogere serum $25OHD_3$ concentratie dan personen die aangaven vitamine D niet te kennen. In totaal gaf slechts 38% van de participanten aan vitamine D te kennen. Echter, onder deze personen, die wel aangeven hadden vitamine D te kennen, bestonden voorts veel misvattingen over zowel de mogelijke bronnen als de werking (effecten) van vitamine D. Hoofdstuk 2.3 en 2.4 richten zich op trendontwikkelingen van de meest voorkomende typen fracturen bij ouderen: wervelfracturen en heupfracturen. Het risico op het optreden van deze beide type fracturen is sterk geassocieerd met osteoporose. Vitamine D deficiëntie is op zijn beurt weer een zeer belangrijke risicofactor voor het ontwikkelen van osteoporose. Hoofdstuk 2.3 beschrijft de prevalentie van heupfracturen in Nederland. De incidentie van

het aantal heupfracturen in Nederland steeg sterk tot ongeveer het midden van de 90-er jaren. Nadien begon de incidentie van heupfracturen geleidelijk te dalen. Er is hierin echter wel een verschil waarneembaar tussen verschillende leeftijdsgroepen. Hoewel de incidentie in relatief jongere leeftijdsgroepen (<80 jaar) daalde, nam de incidentie nog steeds toe in oudere leeftijdsgroepen (>80 jaar). De onderliggende oorzaak van dit verschil is niet bekend. In Hoofdstuk 2.4 wordt het aantal nieuwe wervelfracturen beschreven waarvoor een bezoek aan de Eerste Hulp noodzakelijk is. Over het algemeen neemt het aantal wervelfracturen, in vrijwel alle verschillende leeftijdsgroepen, sterk toe gedurende de laatste tientallen jaren. Echter, ook hier valt op dat het aantal wervelfracturen het sterkst toeneemt bij de oudste-ouderen. De resultaten van deze studies laten zien dat er met name meer aandacht moet zijn voor de preventie van zowel valincidenten en fracturen, vooral in deze oudste leeftijdsgroepen.

In Sectie 3 wordt de relatie tussen vitamine D status en functionaliteit bij ouderen bestudeerd. Hierin wordt er een focus gelegd op spierfunctie, neurologische functie en cardiovasculaire functie. Allereerst wordt in Hoofdstuk 3.1 de associatie tussen vitamine D status en spierfunctie beschreven. In onze studie werd een associatie gevonden tussen vitamine D status en spierfunctie in zowel mannen als vrouwen. Deze studie ontkracht de hypothese dat vitamine D niet belangrijk is voor spierfunctie bij mannen. In Hoofdstuk 3.2 wordt het effect van suppletie met een hoge dosis vitamine D suppletie gedurende 6 maanden onder ouderen wonend in verzorgingshuizen beschreven. De hoge dosis suppletie (1800IE/dag) werd goed verdragen en was effectief voor de behandeling van vitamine D deficiëntie. De behandeling resulteerde in een verbetering van de loopsnelheid en van de scores op de Timed-Up-and- Go-Test (TUGT). Er werd geen effect op spierkracht gevonden. Het waargenomen effect was relatief klein en niet groter dan bij eerdere studies die een lagere dosering vitamine D gebruikten. Hoofdstuk 3.3 beschrijft de associatie tussen vitamine D status en cognitieve functies. In dit hoofdstuk tonen we een associatie aan tussen vitamine D status en cognitieve functie bij patiënten die reeds geheugenklachten hebben.

In Sectie 4 wordt de associatie tussen vitamine D status en cardiovasculaire functie bestudeerd. De resultaten van deze studie duiden erop dat verschillende vasculaire regio's in het lichaam verschillend worden beïnvloed door het

optreden van een vitamine D deficiëntie, wat erop lijkt te wijzen dat de associatie tussen vitamine D en cardiovasculaire functie complex is. Echter, een goed begrip van de samenhang tussen vitamine D status en cardiovasculaire functie is van groot belang. Enkele eerdere studies beschreven een U-vormige associatie tussen vitamine D status en cardiovasculaire functie. Hierbij zou zowel een sterk verlaagde, als een sterk verhoogde serum vitamine D waarde een negatief effect op de cardiovasculaire functie, en gezondheid hebben.

In Sectie 6 worden de belangrijkste bevindingen van dit proefschrift in een breder perspectief geplaatst. De resultaten geven aan dat vitamine D deficiëntie nog steeds erg vaak voorkomt bij ouderen en dat hun kennis omtrent vitamine D matig is. Met betrekking tot functionaliteit bij ouderen toonde dit proefschrift een associatie aan tussen vitamine D en spierfunctie, bij zowel mannen als vrouwen. Hoog gedoseerde vitamine D suppletie onder ouderen in het verzorgingshuis resulteerde in een verbetering van spierfunctie (loopsnelheid en TUGT score). Verder werden er ook associaties gevonden tussen vitamine D status en cognitieve functies, en vitamine D status en diverse cardiovasculaire functies. Vervolgonderzoek is noodzakelijk, met name om de optimale vitamine D status of supplement dosis te bepalen.

DANKWOORD / ACKNOWLEDGEMENTS

Nu, iets meer dan 8 jaar na het starten van mijn promotie traject ben ik toe aan het schrijven van mijn dankwoord. Het is een lange weg geweest die niet altijd even makkelijk was. Toch kijk ik, nu het proefschrift is afgerond, met genoegen terug op deze periode. Zonder alle hulp de afgelopen jaren van veel collega's, vrienden, en familieleden was dit proefschrift niet tot stand gekomen. Ik wil iedereen die mij de afgelopen jaren geholpen heeft, hiervoor hartelijk danken!. Verder geven deze laatste pagina's mij de gelegenheid enkele personen in het bijzonder te noemen.

Geachte professor van der Cammen, beste Tischa, gevat door uw enthousiasme heb ik gekozen voor de ouderengeneeskunde. U heeft dit promotie traject mogelijk gemaakt, wat uiteindelijk heeft geresulteerd in dit proefschrift. Ik heb de afgelopen jaren ontzettend veel van u geleerd, zowel op professioneel als persoonlijk vlak: zo ziet u kansen en mogelijkheden waar anderen dat niet zien, heeft een bijzondere gave om "out of the box" te denken en als voornaamste: geeft u nooit op, ook al is het nog zo lastig. Met veel plezier denk ik terug aan alle congresbezoeken, en alle ontmoetingen met personen waar u mij introduceerde. Ook aan de vele avonden waar we, soms aan de keukentafel, mijn artikelen corrigeerden, denk in met veel genoegen terug.

Geachte dr. Colin, beste Edgar, toen we jou vanuit de geriatrie benaderden met de vraag of je mijn promotie-traject wilde begeleiden stemde je direct in. Je bent al die jaren een onmisbare steun geweest en zonder jou was dit proefschrift er uiteraard niet gekomen. Menigmaal ben je helemaal vanuit het oosten 's lands naar Rotterdam gekomen voor een overleg. Je kritische blik en soms "botte bijl" waren bijzonder scherp. Ook je humor, met niet zelden een licht cynische karakter, heb ik bijzonder gewaardeerd!.

Geachte professor van Leeuwen, beste Hans. Dank dat je mijn promotor wilde zijn. Hoewel de geriatrie aanvankelijk niet direct behoorde tot jouw veld van expertise, was je altijd bereid mee te denken en je in de materie te verdiepen. Verder gebeurde het best wel eens dat ik een deadline niet haalde en was je

altijd bereid tijd voor me vrij te maken. Ik denk met veel genoegen terug aan de gesprekken op jouw kamer waar je altijd in staat was kritische vragen te stellen en mij zo aanspoorde tot verdere verdieping.

Geachte dr. Mattace Raso, beste Francesco. We werken nu al vele jaren samen. Of het nu is op wetenschappelijk gebied, in de kliniek, of op persoonlijk vlak: je bent altijd bereid advies te geven of je hulp aan te bieden. Ik waardeer je steun enorm en dat heeft mij ontzettend geholpen om te komen op de positie waar ik nu ben. Ik hoop nog vele jaren met je samen te werken en te kunnen genieten van je "slechte grappen".

Geachte dr. van der Velde, beste Nathalie. Toen ik begon bij de geriatrie, als opleidings-assistent, en later als promovendus, was jij al bijna in de afrondende fase van je proefschrift. We zijn een tijd kamergenoten geweest op de "duiventil", waar ik van nabij zag dat promoveren soms heel hard werken is, maar ook ontzettend veel leuke en uitdagende kanten met zich mee kan brengen. Ik heb ontzettend veel van je geleerd en ben je dankbaar voor alle hulp de afgelopen jaren. Met name ook voor de momenten dat je er als *"praatpaal/luisterend oor"* was!.

Geachte dr. Hartholt, beste Klaas. Op het moment dat er enkele donkere wolken boven mijn promotie traject hingen (toen in het lab de cellijnen toch niet zo levensvatbaar bleken…) kwam jij als een soort sneltrein voorbij. We hebben samen in korte tijd enkele artikelen geschreven en mede daardoor mijn promotietraject weer op de rit gekregen. Verder uiteraard dank voor alle keren dat je me hebt bijgestaan met statistisch advies of het maken van een figuur!

Geachte Prof. dr. A.J. van der Lely, Prof. dr. P. Patka en Prof. dr. P. Lips, hartelijk dank voor het zitting nemen in de kleine commissie en voor het inhoudelijk beoordelen van mijn proefschrift.

Uiteraard wil ik ook Anke Nijs bedanken. Anke, jij hebt mij als onderzoeksverpleegkundige vanuit de reumatologie ontzettend geholpen bij het verrichten van de interventie studie. Je niet aflatende enthousiasme en doorzettingsver-

mogen werkte bijzonder aanstekelijk. De deelnemers spraken altijd bijzonder enthousiast over je, en velen genoten van je aanwezigheid!.

Uiteraard wil ik ook iedereen van het reuma lab bedanken: Erik, Anne-Marie, Ferry, Sonja, Lisette en uiteraard: Patrick. Patrick, als mede Dordtenaar, bedankt voor alles wat je de afgelopen jaren hebt gedaan! Bij bijvoorbeeld het kweken van cellen, het verrichten van ELISA's en het analyseren van de data. Ik heb veel geleerd van je en ik kijk met heel veel plezier terug naar de tijd op het lab!.

Verder wil ik natuurlijk ook alle bewoners en medewerkers van de zorginstellingen bedanken die ons geholpen hebben de onderzoeken mogelijk te maken. Hierin gaat mijn dank in het bijzonder uit naar Romke van Balen. Romke, doordat jij bereid was ons te helpen met het opstarten van de studie was het uiteindelijk mogelijk hieraan een vervolg te geven.

Ook wil ik alle co-auteurs bedanken die hebben meegeholpen aan het schrijven van de artikelen waarop dit proefschrift is gebaseerd.

Beste vrienden en familie, uiteraard wil ik jullie ook bedanken voor de steun de afgelopen jaren. Met name ook mijn "broertje" voor alle keren dat hij te hulp moest schieten. Ar, bedankt voor alle steun!

Ik ben ontzettend blij dat mijn paranimfen, Rozemarijn en Raymond mij bij willen staan tijdens de verdediging van mijn proefschrift. Rozemarijn, als kamergenoot heb jij de laatste jaren van mijn promotie van nabij meegemaakt. Wat er ook is, ik kan je altijd om hulp vragen, en je komt altijd met een oplossing!. Met name je lijstjes met probleem-oplossende antwoorden zijn een enorme steun geweest de afgelopen jaren.

Raymond, waarschijnlijk ben ik verantwoordelijk voor ontstaan van je eerste grijze haren, toen je mij zo'n 20 jaar geleden al bijles natuurkunde en wiskunde gaf of weer eens de computer moest repareren. Geweldig dat je ook nu mijn paranimf wilt zijn!.

Tot slot Anneke, ik wil je bedanken voor alle steun de afgelopen jaren. Er waren momenten dat je even genoeg had van "vitamine D" maar als het nodig was stond je altijd voor mij klaar. De keren dat je in slaap viel, terwijl ik vol enthousi-

asme mijn review voorlas ter controle op spelfouten, liggen gelukkig ver achter ons…

CURRICULUM VITAE

Christian Oudshoorn was born on October 3rd in Leiden, the Netherlands. After graduating high school at the Huygens Lyceum in Voorburg, he started his medical training at the Erasmus University in Rotterdam in 1998. After completing his medical training he started his residency in internal medicine and geriatrics at the department of internal medicine in 2004 under the supervision of prof. dr. T.J. M. van der Cammen. After completing his residency in 2013, he started working as internist-geriatrician at the department of Internal Medicine, Section of Geriatrics of the Erasmus MC University Medical Center. The studies on vitamin D which resulted into this dissertation were performed during this period.

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PHD PORTFOLIO

Name PhD student	Christian Oudshoorn
Erasmus MC Department	Internal Medicine, Section of Geriatric Medicine
PhD period	2007-2016
Promotoren	Prof. dr. J.P.T.M. van Leeuwen Prof. dr. T.J.M. van der Cammen
Co-promotor	dr. E.M. Colin

Summary of PhD-training and teaching

PhD training
General courses

- Teach the teacher II
- Classical Methods for Data-analysis, NIHES course in Biostatistics. Rotterdam, the Netherlands, 2007
- Summer Course (NIHES), NIHES, 2007

Specific courses (Medical training, research)

- European Academy for Medicine of Aging (EAMA IX), Martigny, Switzerland: 2011-2012

Presentations

- Various presentations at research seminars and symposia of the Erasmus University Medical Center

Presentations (inter)national conferences
Oral

- 2009: VDR haplotype blocks and the incidence of Alzheimer dementia: Gemeinsamer Österreichisch-Deutscher Geriatriekongress (Vienna)
- 2010: Effects of aging on vitamin D and calcium homeostasis: International Nursing Congress (Rotterdam)

- 2011: Effects of vitamin D supplementation on neuromuscular functioning: IAGG (Bologna)
- 2013: Effecten van vitamine D suppletie op spierkracht en mobiliteit: Gertia-triedagen (Den Bosch)
- 2013: Vitamin D and neuromuscular function: IAGG (Venice)
- 2013: Vitamine D en spierfunctie: 1e Nationaal Valsymposium (Amsterdam)
- 2014: Vitamine D en veroudering: 7e Rotterdamse Internistendag (Rotterdam)

Poster

- 2010: Knowledge on vitamin D and calcium in older person: EUGMS (Dublin)
- 2011: Trends in osteoporotic fractures in the Netherlands: Gemeinsamer Österreichisch-Deutscher Geriatriekongress (Vienna)
- 2016: Kennis omtrent de bronnen en effecten van vitamine D: een systematische review van de literatuur: Geriatriedagen (Den Bosch)

Other conferences attended

- 2014: Vitamin D and Human Health: from Gamate to Grave (London)

Lecturing

- Lecturing for medical students at the Erasmus University
- Lecturing for residents internal medicine and residents geriatric medicine
- Lecturing for residents psychiatry and for residents gastroenterology

Supervising

- Supervising student literature project on vitamin D: Defining the optimal vitamin D status for muscle function and bone health. M. van Marrewijk, K. Bokent, R. Verdonschot

Prizes:

- Winner: "Wetenschapsprijs Nederlandse Vereniging voor Klinische Geriatrie 2014"