Vitamin D *and* muscle function *in* older people

Hennie Janssen

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Vitamin D and muscle function in older people

Vitamine D en spierfunctie in ouderen

(met een samenvatting in het Nederlands)

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Voor Jolien en Tim

Ga maar op mijn schouders staan en kijk de wijde wereld rond,
wees niet bang om te vallen want ik zal er zijn om je te vangen

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General introduction

General introduction

Aging, even in apparently healthy older people, is accompanied by a reduction in muscle mass and muscle strength[1]. When the gradual loss of muscle strength drops below a certain threshold, functional mobility is affected[2]. An estimated 20-30% of older people experience functional impairment and disability[3]. Therefore preserving muscle strength in this population is of major importance.

Sarcopenia

The term sarcopenia (in Greek *sarx* means flesh, and *penia* means loss of) is used to denote loss of muscle mass occurring with advancing age. Although diagnostic criteria for age-related sarcopenia are subject of debate, a definition was postulated in 2010 in which sarcopenia is considered present when there is a loss of muscle mass 2 standard deviations below the sex-specific mean in a young population and walking speed is below 0.8 m/s[4]. The estimated prevalence of sarcopenia is, depending on the definition used, 5-13% for those above 60 years of age, increasing to about 50% in people over the age of 80 years[5].

Sarcopenia is characterized by an overall loss of muscle mass of about 25% between the ages of 30 and 80 years, although changes are not uniformly distributed in the body, and most pronounced in the lower limb muscles[6]. Evidence from biopsies in the m. vastus lateralis indicates that loss of muscle mass in older people is due to both a loss of number and size of muscle fibres[7]. In addition to a loss of muscle mass, muscle composition changes with advancing age due to motor unit denervation and subsequent renervation[8] with fibre type grouping and a particular loss of type II (fast twitch) fibre area, and also infiltration of fat[9,10].

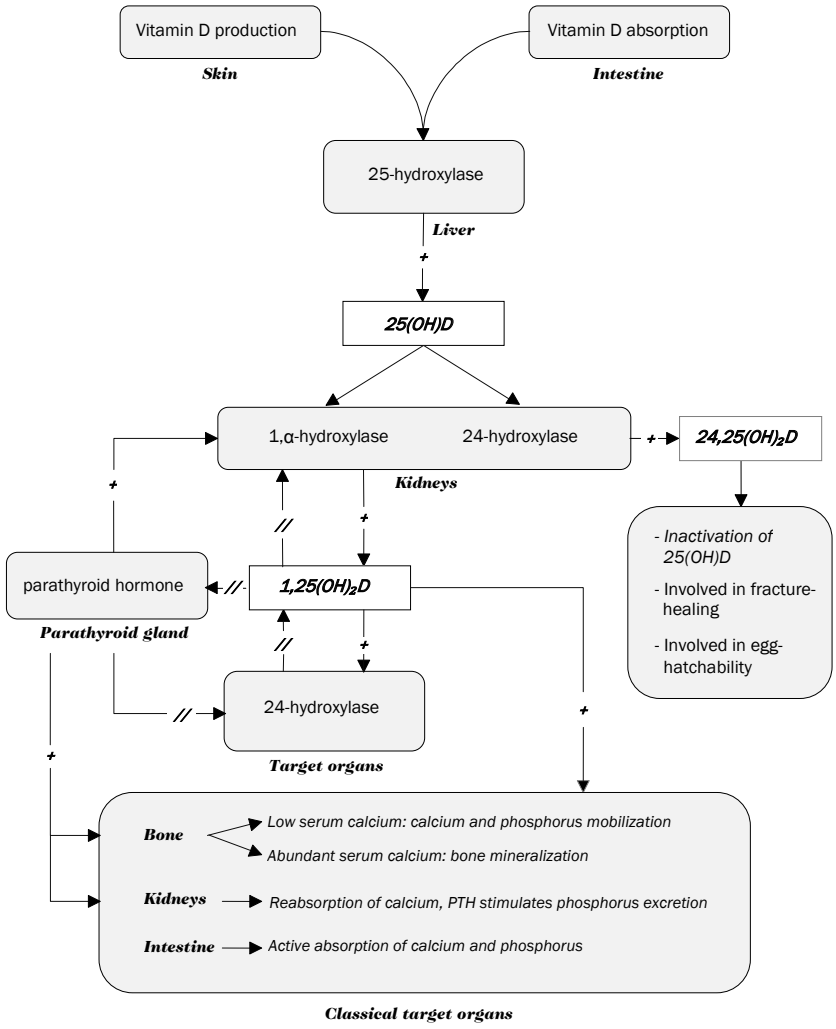
Whether the age-related decrease in muscle mass in itself is associated with functional impairment is controversial[11-13] and it appears that the combination of sarcopenia with obesity (sarcopenic obesity) is most detrimental for physical functioning[14]. The cause of sarcopenia is multifactorial, and includes reduced physical activity, inadequate nutrient intake, decrease of sex hormones[15], increase of pro-inflammatory cytokines[16], reduced synthesis of mitochondrial proteins[17] and also vitamin D deficiency[18] has been implicated as one of the potential causes of the age-related muscle loss that is sarcopenia[19].

Vitamin D

When vitamin D was first discovered it was recognized as an essential nutrient and classified as one of the fat-soluble vitamins. Later on it was discovered that the body is capable of producing vitamin D and that a vitamin D endocrine system exists, generating the active vitamin D steroid hormone 1α,25-dihydroxyvitamin D[21]. In humans two sources of vitamin D exist. First, and most important, is the production of vitamin D in the skin under the influence of ultraviolet light. In addition to the photoconversion in the skin, vitamin D can be obtained from the diet, by ingesting vitamin D containing products (i.e. fatty fish, vitamin D fortified milk or margarine, and the use of (multi)vitamins), which are absorbed in the small intestine. Ingested vitamin D is transported by the lymphatic system into the venous system. In the serum, bound to a protein, vitamin D is taken up by adipose tissue for storage or by the liver for further metabolism. In the liver, vitamin D is hydroxylated, resulting in 25-hydroxyvitamin D. This vitamin D metabolite is transported to the kidneys, where a second hydroxylation results in 1α,25-dihydroxyvitamin D, the active metabolite of vitamin D[22]. Although 1α,25-dihydroxyvitamin D is predominantly produced in the kidneys, 25-hydroxyvitamin D hydroxylation is also found in extra-renal sites, such as the skin, the gastro-intestinal tract, the reproductive organs, the central nervous system, the immune system and in osteoblast and osteoclasts[23]. The production and subsequent degradation of 1α,25-dihydroxyvitamin D in the kidneys is under tight metabolic control by various feedback systems (Figure 1). So even if there is a substrate shortage the concentration of this metabolite will remain within reference limits for a long time. This is in contrast to the concentration of 25-hydroxyvitamin D, which is directly influenced by the supply, and constitutes a storage facility. This is why it is generally agreed that vitamin D status is most accurately reflected by the serum 25-hydroxyvitamin D concentration.

The active vitamin D metabolite 1α,25-dihydroxyvitamin D exerts its action on distant target sites via a nuclear or a membrane-bound vitamin D receptor and has a role in a great variety of (patho)physiological functions. Vitamin D has a crucial role in the maintenance of bone metabolism and calcium homeostasis, but is also involved in the occurrence of malignancy, autoimmune disease, mood disorders, diabetes, cardiovascular disease and muscle weakness[24]. Vitamin D deficiency is associated with a deep musculoskeletal pain and weakness, predominantly of the proximal muscle groups and histopathologically, muscle atrophy particularly of type II fibres has been described[25-29].

Figure 1 Feedback and regulation in vitamin D metabolism



25(OH)D = 25-hydroxyvitamin D; 1,25(OH)$_2$D = 1,25-dihydroxyvitamin D; 24,25(OH)$_2$D = 24,25-dihydroxyvitamin D; PTH = parathyroid hormone; '+' = production, stimulation; '//' = inactivation, inhibition.

With advancing age the risk of vitamin D deficiency increases due to less sun exposure (in case of mobility impairment or more skin coverage when going outside) and also diminished skin thickness causing less production of vitamin D in the skin. In addition to reduced vitamin D production in the skin, decreased dietary intake of both vitamin D and calcium, impaired hydroxylation in the liver (in liver disease and due to medication such as anticonvulsants) and in the kidneys (in case of impaired renal function) may further compromise vitamin D status in older people[30,31]. Vitamin D deficiency is common in older people with two-thirds of community-dwelling older women in Northern Europe having an insufficient vitamin D status in the winter[20]. Because it is common in older people and it has been associated with muscle weakness, vitamin D supplementation in order to preserve muscle strength and functional mobility in this population, could therefore be an important intervention.

Aim of this thesis

The aim of this thesis is:

- To investigate the determinants of vitamin D status in middle-aged and older people.
- To determine the relation between vitamin D status and muscle function in middle aged and older people.
- To assess the effect of vitamin D supplementation in preserving muscle function and functional mobility in older people.

Outline of this thesis

In **Chapter 2** the relation between vitamin D deficiency, muscle function and falls in older people is reviewed in order to determine the possible benefits of vitamin D supplementation for improvement of muscle strength and functional ability in this population.
In **Chapter 3** contributors of functional mobility and fall occurrence in ambulatory women referred to a geriatric outpatient clinic are described.
The contribution of gender, age, season, life style factors, hormonal changes and health related factors on vitamin D status in a population of independently living middle-aged and older men and women with an age span of 40 to 80 years is presented in **Chapter 4**.
In **Chapter 5** we investigated the relation between serum 25-hydroxyvitamin D and muscle mass and function in independently living middle-aged and older people.

In **Chapter 6** the results of a randomized, double-blind, placebo-controlled trial on the effect of vitamin D and calcium supplementation as compared with calcium mono-therapy on muscle strength, power and functional mobility in vitamin D insufficient female geriatric patients are presented.

In **Chapter 7** the main findings of this thesis are summarized, and the overall effect of vitamin D on muscle function is discussed, with implications for clinical practise and future research.

A summary completes this thesis.

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02

Vitamin D deficiency, muscle function and falls in older people

Hennie CJP Janssen, Monique M Samson, Harald JJ Verhaar

American Journal of Clinical Nutrition 2002;75:611–5

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Abstract

An inadequate serum vitamin D status is commonly seen in older people as the result of various risk factors interacting in this population. Apart from the well-known effect on bone metabolism, this condition is also associated with muscle weakness, predominantly of proximal muscle groups. Muscle weakness below a certain threshold will affect functional ability and mobility, which puts an older person at increased risk of falling and fractures. Therefore, we wanted to determine the rationale behind vitamin D supplementation in older people to preserve and possibly improve muscle strength and subsequently functional ability.

From experimental studies it was found that vitamin D metabolites directly influence muscle cell maturation and functioning through a vitamin D receptor (VDR). Vitamin D supplementation in vitamin D deficient, older people improved muscle strength, walking distance and functional ability. In addition, a reduction in falls and non-vertebral fractures was observed upon vitamin D supplementation in this population.

In healthy, older people, muscle strength declined with age, and this could not be prevented by vitamin D supplementation. In contrast, severe comorbidity might affect muscle strength in such a way, that restoring vitamin D status has limited effect on functional ability.

Additional research is needed to further clarify to what extent vitamin D supplementation can preserve muscle strength, and prevent falls and fractures in older people.

Introduction

Aging, even in healthy older people, is accompanied by a reduction in muscle mass and muscle strength[1-3]. Gradual loss of muscle strength will (below a certain threshold) result in functional impairment[4,5], dependence in the performance of daily activities[6,7], with an increased risk of falling and non-vertebral fractures[8]. Therefore preserving muscle strength in this population is of major importance.

Vitamin D deficiency is a condition associated with muscle weakness[9], and is commonly encountered in older people[10]. Due to various risk factors (i.e. decreased dietary intake, diminished sunlight exposure and reduced skin thickness, impaired intestinal absorption, impaired hydroxylation in the liver and kidneys), older people are prone to develop vitamin D deficiency[11-13]. In 824 elderly people (age > 70 yr.) from eleven European countries, 36% of men and 47% of women had wintertime serum 25-hydroxyvitamin D_3 (25(OH)D) concentrations below 30 nmol/l[14].

Muscle weakness due to vitamin D deficiency is predominantly of proximal muscle groups, with a feeling of heaviness in the legs, easy tiring, difficulty in mounting stairs and rising from a chair, which is reversible upon supplementation[15-18]. Histopathologically, muscle atrophy particularly of type II fibers has been described[17,19,20] .

In this review we focused on the relation between vitamin D deficiency, muscle function and falls in older people, in order to determine the benefits of vitamin D supplementation for improvement of muscle strength and functional ability in this population.

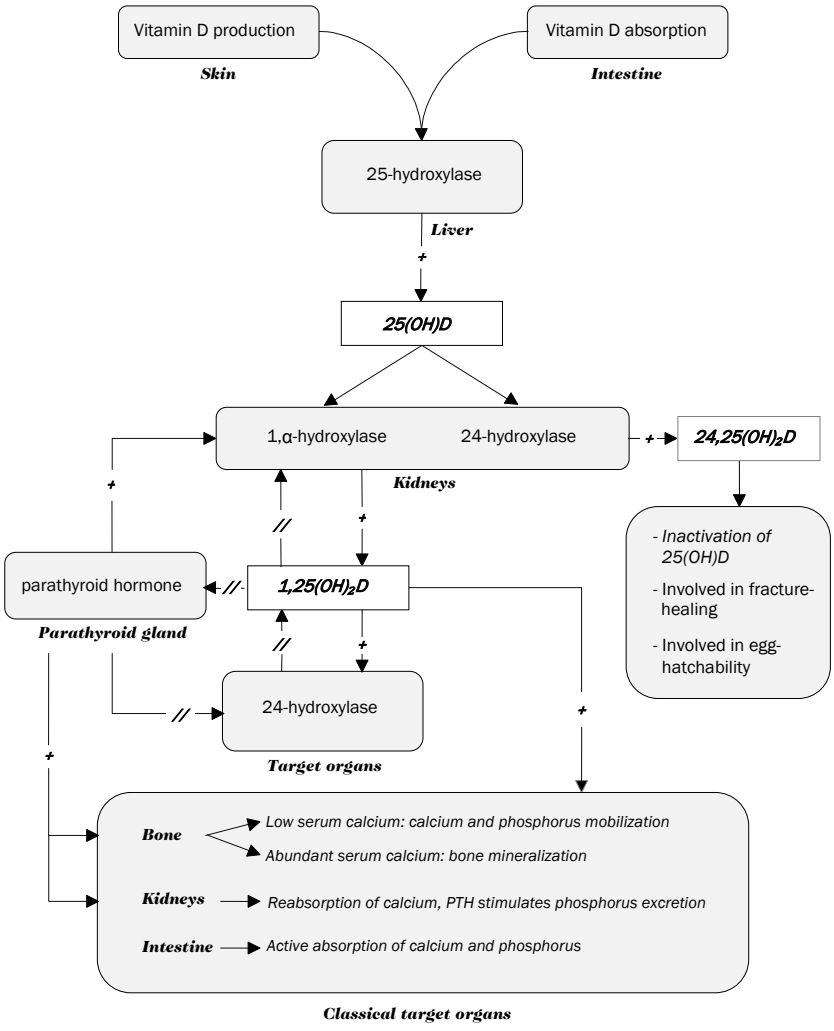
Vitamin D metabolism

In the skin, under influence of ultraviolet radiation, 7-dehydrocholesterol is photoconverted to previtamin D_3, which is converted to vitamin D_3 (cholecalciferol). In the serum, bound to a vitamin D binding protein (DBP), vitamin D_3 is transported to the liver where it is hydroxylated resulting in 25-hydroxyvitamin D_3 (25(OH)D). In the kidneys, 25(OH)D is further metabolized to 1α,25-dihydroxyvitamin D_3 (1,25(OH)D), the biologically active form of vitamin D[21]. Its production and subsequent degradation is under tight metabolic control by various feedback systems which are presented in Figure 1[22-28].

In addition to the photoconversion in the skin, vitamin D can be obtained from diet, through ingestion of vitamin D containing products (e.g. fatty fish), vitamin D fortified milk or margarine, and the use of (multi)vitamins. The vitamin D ingested via this route is metabolized in the same manner as the endogenously produced vitamin D.

Because 1,25(OH)D exerts its influence on distant target tissue, mediated by a vitamin D receptor (VDR), it is considered to be a hormone rather than a vitamin[29]. The

Figure 1 Feedback and regulation in vitamin D metabolism



25(OH)D = 25-hydroxyvitamin D; 1,25(OH)$_2$D = 1,25-dihydroxyvitamin D; 24,25(OH)$_2$D = 24,25-dihydroxyvitamin D; PTH = parathyroid hormone; '+' = production, stimulation; '//' = inactivation, inhibition.

serum value of 25(OH)D is a factor 1000 higher than the serum 1,25(OH)D value, and this substrate excess constitutes a storage facility, in line with other steroid hormones. Although it is generally agreed that vitamin D status is most accurately reflected by the serum 25(OH)D concentration, evidence regarding the adequate serum concentration is inconclusive. An elevated serum parathyroid hormone concentration is a commonly used marker to define vitamin D deficiency. However, in order to prevent secondary hyperparathyroidism different corresponding 25(OH)D concentrations have been reported, varying from 20 - 40 nmol/l up to 122 nmol/l[30-34]. Alternatively, a gradual scale was proposed in which hypovitaminosis D is considered when 25(OH)D < 100 nmol/l (40ng/ml), vitamin D insufficiency if 25(OH)D < 50 nmol/l (20 ng/ml), and vitamin D deficiency with a 25(OH)D concentration < 25 nmol/l (10 ng/ml)[35].

By using elevated serum parathyroid hormone as a means for defining vitamin D deficiency, it should be recognized that physical inactivity increases bone turnover, and serum calcium concentration, thereby preventing elevation of serum parathyroid hormone, even in the presence of vitamin D deficiency[36]. In addition, caution is needed when comparing results from studies using different assay techniques to determine serum 25(OH)D_3[7].

Apart from maintaining body calcium homeostasis by affecting classical target organs (intestine, kidney, bone, parathyroid gland), other target sites for vitamin D metabolites have been identified, i.e. skin, muscle, pancreas, immune system, hematopoetic system, and reproductive organs, and new actions have been discovered[21].

Muscle as a target site for vitamin D metabolites

Birge and HaddaD[8], in the mid-seventies, were the first to demonstrate that 25(OH)D directly influenced muscle phosphate metabolism in diaphragms from vitamin D-deficient rats. Since then, several studies have shown that vitamin D metabolites affect muscle cell metabolism through various pathways. It is beyond the scope of this paper to present these mechanisms in detail, which are thoroughly described elsewhere[39,40].

Vitamin D metabolites have been found to affect muscle metabolism in three ways:

① by mediating gene transcription;

② through rapid pathways not involving DNA synthesis;

③ by the allelic variant of the vitamin D receptor.

Both in animal models[41] and in humans[42,43], a vitamin D receptor (VDR) has been found in skeletal muscle cells, which specifically binds 1,25(OH)D. After transportation

to the nucleus, this ligand-receptor-interaction is modulated by various transcription factors and biochemical processes, resulting in a final transcription complex[21]. In cultured myoblasts this genomic pathway has been found to influence muscle cell calcium uptake, phosphate transport across the muscle cell membrane, phospholipid metabolism and to mediate cell proliferation and subsequently differentiation into mature muscle fibers[40,43-46].

Vitamin D supplementation induced rapid changes in calcium metabolism of the muscle cell, that cannot be explained by a (slow) genetic pathway. Recent evidence indicates that 1,25(OH)D, possibly through a vitamin D membrane receptor[47,48], acts directly on the muscle cell membrane. Upon 1,25(OH)D binding, several (interacting) second-messenger-pathways were activated in the muscle cell, resulting in enhanced calcium uptake (within minutes) both through voltage-dependent-calcium-channels[49,50], and calcium-release-activated-calcium-channels[51].

Finally, muscle strength appears to be influenced by the genotype of the VDR present in the muscle cell. With the use of specific restriction endonucleases, several VDR polymorphisms have been determined. In non-obese older women a 23 % difference in quadriceps strength, and 7% difference in grip strength between the two homozygote types of a restriction site were found[52].

Vitamin D and muscle function

In recent years, various cases of both young[15,17,19] and older adults[16,53] have been described in which prolonged vitamin D deficiency was associated with severe muscle weakness, often leading to marked disability[15,16], that improved within several weeks of vitamin D supplementation. However, few studies have been conducted in which muscle strength was objectively quantified in relation to vitamin D status in older people.

In an older population (65 - 95 yr.), in which 12% of women and 18% of men had serum 25(OH)D concentration < 30 nmol/l, a significant correlation was found between vitamin D metabolites and leg extension power[54]. This is in agreement with the study of Mowé et al.[55], in which the association between serum vitamin D metabolites and muscle function was examined. In 349 older people ($\geq$ 70 yr.), of which 246 were recently hospitalized, serum 25(OH)D concentration was significantly lower in subjects with less hand grip strength, in those unable to climb stairs, without any outdoor activity, and with episodes of falling in the previous month[55]. In addition, a low serum 25(OH)D concentration ($<$ 40 nmol/l) was associated with reduced

handgrip strength and walking distance in 63 community-dwelling elderly (82.5 ± 5.4 yr.)[56]. However, from cross-sectional studies a causal relation cannot be inferred. Other conditions may cause muscle weakness and impair mobility, thereby preventing an older person from going outside. Nevertheless, evidence from intervention studies does indicate causality.

Muscle strength and mobility was measured in 27 older women (on average 76 years). Six months treatment with 0.5 ug alphacalcidiol per day significantly improved both knee extension strength and walking distance in the vitamin D deficient (25(OH)D < 20 nmol/l) women, as opposed to the vitamin D replete (thus receiving no therapy) control subjects who showed no improvement[57]. In frail older people, vitamin D and calcium supplementation significantly improved 'Time taken to dress'[58], and functional ability measured with a Frail Elderly Functional Assessment Questionnaire[59]. In contrast, upon supplementation with 9000 U vitamin D2, no significant difference in ADL performance was found in patients, admitted on a geriatric ward for a longer period, as compared with a placebo group[60]. However, the high prevalence of severe comorbidity present in this population likely affected functional performance as well. In a healthy, vitamin D replete, older population (70 - 90 yr.), no correlation was found between serum 1,25(OH)D concentration and knee extension strength, although both declined with age[61]. This is in accordance with the study of Grady et al.[62] in which 98 healthy, mostly vitamin D replete, volunteers (> 69 yr.) were treated with 0.5 μg 1,25(OH)D daily or a placebo for 6 months, after which no significant differences in knee extension or flexion strength were noticed. Although muscle strength declined by 1.6%/yr in this population, serum 1,25(OH)D concentration remained stable with age, increasing only moderately after 6 months treatment[62].

Vitamin D and falls

On average a third of older people experience at least one fall per year[63-65], of which approximately 6 - 7 % result in a fracture[63,65].

Vitamin D and calcium supplementation significantly reduced the number of hip fractures (by 43 %, P = 0.043) in a French, female, nursing home population (mean age 84 ± 6 yr.), as compared with placebo-control subjects. Bone mineral density improved significantly in the active treatment group as compared with the placebo-control group (respectively + 2.7 % and - 4.6 %, P < 0.001)[66]. Although not stated by the authors, a reduction in the number of falls in the active treatment group as compared with the control-group, might have contributed to the reduced hip fracture

incidence in this population[56].

Indeed, vitamin D deficiency has been reported to affect predominantly the weight-bearing antigravity muscles of the lower limb, necessary for postural balance and walking[67], and a significant correlation between serum 25(OH)D concentration and the occurrence of falls in older people has been presented[55,68]. Furthermore, eight weeks supplementation with vitamin D and calcium of 148 older women with a serum 25(OH)D concentration below 50 nmol/l, resulted in a decrease in body sway of 9 % ($P < 0.05$), and a reduction in the mean number of falls per subject during a one-year follow-up, as compared with the control-group who received calcium monotherapy (0.45 vs. 0.24, < 0.05)[69]. In contrast, 2 years of 400 IU vitamin D_3/day did not reduce the number of falls in a Dutch population (> 70 yr.) as compared with a placebo control group[65], and three years of vitamin D and calcium supplementation did not reduce fall incidence in a healthy older (> 65 yr.) Boston population as compared with placebo treatment[70]. In the STOP IT trial, 489 women (mean age 71 yr.) were randomly assigned to estrogen, calcitriol, a combination of both, or a placebo for three years. Although the increase in bone density was twice as large on estrogen therapy as compared with calcitriol, subjects receiving calcitriol experienced less fractures through falls as compared with the estrogen group (respectively, odds ratio 0.78 and 0.94)[71]. Thus, indicating another mechanism, apart from improving bone density, in the prevention of non-vertebral fractures.

Discussion

The aim of this review was to clarify the impact of an inadequate vitamin D status on muscle function in older people, and to determine the rationale behind vitamin D supplementation in order to preserve muscle strength and functional ability. Comparing results from various studies is somewhat hampered by differences in subject demographics, study design and outcome parameters. Nevertheless, evidence indicates that in older people muscle function is affected by an inadequate vitamin D status[54-56]. Supplementation in this population improved muscle strength, walking distance, functional ability[57-59], and body sway[70]. Positively affecting these parameters, in addition to the improvement of bone density[67,70], provides an explanation to the association between vitamin D supplementation and the reduction in the number of falls and nonvertebral fractures in older people[69,71].

However, vitamin D deficiency is merely one condition affecting muscle function in older people[73,74], which is illustrated by the fact that even in healthy, vitamin D re-

plete, older people muscle strength declined with age[61], that could not be prevented by vitamin D supplementation[62,75]. Moreover, severe comorbidity (and subsequent immobility) may cause muscle weakness and functional impairment, which cannot be improved by treating a coexisting vitamin D deficiency[60].
Experimental studies have shown that muscle tissue is a direct target site for vitamin D metabolites and offer biochemical evidence for the weakness associated with vitamin D deficiency[38]. Although 1,25(OH)D is considered the active metabolite affecting target sites, including muscle[41], clinical studies reported a relation between serum 25(OH)D and muscle strength[55,68] and functional ability[59]. Two mechanisms might explain these findings. First, the serum 25(OH)D concentration is a 1000-fold higher than serum 1,25(OH)D, and this might result in competitive binding of the two D-metabolites on the vitamin D receptor[76]. Another explanation might be the fact that peripheral tissues, previously recognized as target sites for vitamin D metabolites, were found to express the mitochondrial enzyme 1α-hydroxylase[77]. Activation of 25(OH)D locally in target tissues may be involved in regionally controlled cell function[78].

In conclusion, vitamin D deficiency is a condition that may cause muscle weakness in an older person. Although only a few intervention studies have been conducted in older people, the available evidence indicates that vitamin D supplementation is of benefit in high risk groups (i.e. frail, mostly homebound older people), to preserve muscle strength and functional ability. Additional research, preferably by means of controlled randomized trials, is needed to confirm these findings.

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Notes

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Strength, mobility and falling in women referred to a geriatric outpatient clinic

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Abstract

Background and aims

Mobility impairment and falling have a multifactorial etiology in frail older people. Muscle weakness is one of the risk factors and is accessible to intervention. The aim of this study was to determine the most important contributors of mobility and indicators of fall occurrence in women referred to a geriatric outpatient clinic.

Methods

Mobility was assessed using the Timed 'Get-Up-and-Go' test (TGUG) and the modified Coopertest (COOP). Falling was assessed retrospectively and isometric knee extension force was measured using fixed dynamometry. Habitual physical activity was quantified using a questionnaire for the elderly. Height, weight, medical conditions and current medication were recorded.

Results

Isometric knee extension strength and habitual physical activity, which consisted predominantly of household work, were independent variables of performance on TGUG and COOP and together explained 57% of the variance in TGUG (r 0.75, $p < 0.001$), and 64% of that in COOP (r 0.80, $p < 0.001$). Age, total number of medical conditions, and presence of cardiovascular disease were not significant in the model.

Women in the lowest tertile of knee extension strength had a significantly higher probability of falling (0.75, 95% CI 0.56 - 0.91) compared with women in the highest tertile (0.27, 95% CI 0.14 - 0.50).

Conclusions

Knee extension strength remains a strong determinant of mobility and fall occurrence in women referred to a geriatric outpatient clinic. Performing light to moderate household work remains independently associated with functional mobility.

Introduction

Quantifying muscle strength and mobility impairment in older people is important because it is associated with dependence in activities of daily living[1], lower self-rated health[2] and increased risk of falling[3]. Older people at the highest risk of recurrent falling are those who are mobile but unstable, while people at the extremes of the mobility spectrum, i.e. very immobile or stable, are at the lowest risk[4]. Moreover, if currently non-disabled older people perform poorly on mobility tests, this predicts future disability[5], hospitalization[6] and mortality[7]. Thus, reduced mobility performance is an early indicator of those older people who are most vulnerable to future disablement.

The cause of impaired mobility is often multifactorial in older people[8]. Even in apparently healthy aging, loss of mobility is observed[9,10], due to inevitable degenerative changes in various organ systems[11], sedentary life-style, and particularly changes in muscle mass, muscle quality, and subsequently strength (sarcopenia)[12]. Apart from this, multiple medical conditions and use of medication in frail older people also affect mobility, i.e., by causing pain, disturbing balance or aggravating age-related strength loss[13].

Evidence from intervention studies indicates that loss of strength and mobility is preventable and reversible to some extent[14,15], even in very old people[16]. Older people referred to a geriatric outpatient clinic, although ambulatory, constitute a vulnerable group in which mobility impairment and a history of falling is frequently observed. Therefore, in the current study we aimed at determining the most important contributors of functional mobility and fall occurrence in ambulatory women referred to a geriatric outpatient clinic.

Methods

This cross-sectional study was carried out as part of a randomized controlled trial on muscle strength, functional mobility and quality of life in female geriatric patients with vitamin D insufficiency. Over a 2-year period (May 2000 until June 2002), women attending the outpatient clinic of the Department of Geriatric Medicine at the University Medical Center Utrecht, The Netherlands, were included if they were > 65 years of age, able to walk, follow simple instructions, and had a serum 25-hydroxyvitamin-D_3 level between 20 and 50 nmol/L. Ninety-one eligible women were approached, to yield 70 subjects who agreed to participate (number based on a power calculation). Reasons for refusal were: no interest in research, feeling unable to come to the

hospital, or already having too much medication or diagnostic testing. All subjects gave their written informed consent. This study was approved by the Ethics Committee of the University Medical Center, Utrecht, The Netherlands. All measurements were carried out by the principal investigator (first author).

Assessments

Subject demographics were obtained from medical records, and height and weight were recorded. Isometric knee extension strength (IKES) was measured with the subject seated in an adjustable, straight-backed chair with the lower leg unsupported and the hip and knee flexed in a 90° angle[17]. Peak values for left and right were averaged for analysis. To account for differences in body weight and height, average knee extension strength was corrected for Body Mass Index (BMI = kg/m ^{2}) in analysis. Mobility was measured with the Timed "Get Up and Go" test (TGUG)[18], using a chair with a built-in timer, and the Modified Cooper test (COOP)[19] , recorded as the maximum walking distance (in meters) in 2 minutes. The occurrence of a fall[20] was assessed by asking the patient and an accompanying relative if a fall had happened in the previous 6 months. Habitual physical activity was measured with an interview-administered questionnaire for the elderly[21]. Activities in three domains (household, sports, leisure time) were determined and combined to give an overall physical activity score.

Statistical analysis

Simple scatter plots and curve estimation were used to select the model which best explained the relation between performance on the mobility tests and independent variables. Significant determinants of mobility were put together in a model using multiple stepwise linear regression and univariate analysis of variance. Student's t- test and the Chi-square test were used to compare independent samples. Point estimates with 95 % confidence intervals (CI) were made to determine the probability of falling. Data are presented as means $\pm$ 1 SD, unless otherwise stated. P-values are considered significant if < 0.05. Analysis was performed using the SPSS (version 9.0) statistical package (SPSS, Chicago, IL).

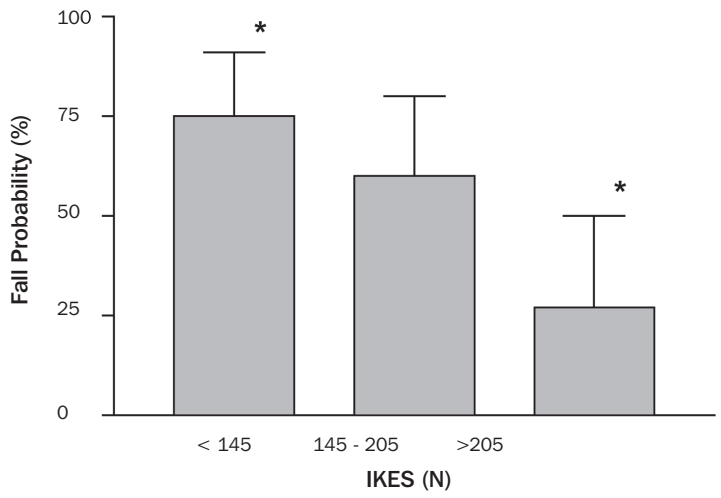
Results

Seventy women with a mean age of 80.8 ± 6.7 years were included. Patient characteristics are presented in Table 1. The average number of medication use was 5.0 ± 3.3, 41% using benzodiazepines. The mean physical activity score was 3.1 ± 2.9, of which the household subscore contributed most (a median of 72%). More than half the subjects (54%) used a walking aid, i.e., cane or walking frame, when going outside. The use of a walking aid was significantly associated with number of medical conditions ($p = 0.01$), and presence of cardiovascular disease ($p = 0.03$).

Because the relation between TGUG and independent variables was best explained by an exponential model, TGUG was transformed to its natural logarithm in further analyses. Knee extension strength and age were linearly associated with COOP. In analysis of variance, only knee extension strength ($p = 0.004$), habitual physical activity ($p = 0.001$) and use of a walking aid ($p < 0.001$) remained significantly related to performance on TGUG and COOP. Age, number of medical conditions, and presence of cardiovascular disease were non-significant in the model. Knee extension strength and habitual physical activity together explained 57% of the variance in TGUG (r 0.75, $p < 0.001$) and 64% of that in COOP (r 0.80, $p < 0.001$).

Thirty-seven women (53%) had experienced at least one fall in the previous 6 months (Table 2). Subjects who used a walking aid ($p < 0.01$) or benzodiazepines ($p < 0.05$) had a significantly higher probability of having experienced a fall compared with those who did not. Isometric knee extension strength and performance on the TGUG and COOP were divided in tertiles. Women with knee extension strength below 145 N (lowest tertile) had a significantly higher probability of a fall (0.75, CI 0.56 - 0.91) compared with women who had knee extension strength above 205 N (0.27, CI 0.14 - 0.50) (Fig. 1). If time needed to complete TGUG was above 15 seconds, the average probability of a fall increased to 74%, compared with a 40% risk of falling, if time to complete TGUG was below 15 seconds.

Figure 1 Fall probability (with 95% CI) for tertiles of isometric knee extension strength (IKES) in Newton (N)



** indicates significant difference ($p < 0.05$) in fall probability between lowest and highest tertiles*

Table 1 Patient characteristics of women (n = 70) referred to a geriatric outpatient clinic

	N	Mean (SD)	Range
Age (yrs)	70	80.8 (6.7)	66 - 95
Body Mass Index (kg/m2)	70	26.4 (4.8)	18 - 41
Walking aid	38		
Falling in previous 6 months	37		
No. of current medications	70	5.0 (3.3)	0 - 13
Use of benzodiazepines	29		
No. of medical conditions	70	2.4 (1.3)	0 - 6
Cardiovascular disease	35		
Chronic venous insufficiency	16		

Table 1 Continued

	N	Mean (SD)	Range
Chronic obstructive pulmonary disease	13		
Cerebrovascular disease	15		
Dizziness	11		
Peripheral neuropathy	4		
Lower extremity arthritis	18		
Visual impairment	8		
MMSE score < 24	16		
Depression	23		
Physical activity score	70	3.1 (2.9)	0 - 12.8
Knee extension strength (N)	67	180.3 (62.3)	42.0 - 367.0
Timed Get Up and Go test (s)	70	13.6 (8.4)	4.4 - 43.6
Modified Cooper test (m)	69	120.7 (41.9)	37 - 229

Table 2 Comparison of women who fell with women who did not. Numbers are means with standard deviations in brackets

	Fallers	Non-fallers	P
Age (yrs)	81.8 (7.1)	79.9 (6.0)	0.09
BMI (kg/m2)*	26.7 (4.9)	26.1 (4.6)	0.31
Medical conditions	2.6 (1.4)	2.2 (1.3)	0.09
MMSE score†	26.0 (5.8)	27.5 (3.4)	0.08
No. of medications	5.0 (3.0)	5.1 (3.6)	0.43
IKES (N)‡	158.1 (51.9)	204.6 (64.5)	0.001
TGUG (s)||	15.2 (8.7)	11.8 (7.8)	0.045
COOP (m)¶	105.8 (39.2)	138.0 (38.7)	0.001
Physical activity score	2.7 (3.1)	3.4 (2.8)	0.16

** BMI : Body Mass Index; † MMSE : Folstein Mini Mental State Examination; ‡ IKES : Knee extension strength, as measured with fixed dynamometer; || TGUG : Timed get Up and Go test; ¶ COOP : Modified Cooper test. P is one-sided*

Discussion

In this sample of ambulatory women referred to a geriatric outpatient clinic, we found that both knee extension strength and physical activity remained independently associated with mobility. The relation between TGUG and knee extension strength was best explained by a non-linear model, in which the influence of knee extension strength on TGUG performance gradually decreased as muscle strength improved. TGUG combines chair stand and gait performance, and both have been reported to be non-linearly related to knee extension strength[22].

In contrast, isometric knee extension strength remained linearly associated with COOP throughout the measured strength range. Two- and six-minute walking tests were initially designed to measure exercise tolerance in respiratory disease[19], but recent studies show that they can also be used as a measure of overall mobility[23]. In healthy older people, quadriceps force played no independent role in walking performance, after correcting for age, sex, height and weight[24]. However, in frail older people, lower extremity strength remained an important predictor of walking ability[25]. Our results are partly consistent with the latter study, although age was not an independent predictor of mobility after controlling for muscle strength and habitual physical activity.

In our study, the use of a walking aid was significantly associated with functional mobility, while number of medical conditions and presence of cardiovascular disease were not. In frail older people, the use of a walking aid is the net result of various conditions affecting strength, balance, coordination or endurance, and may be a marker of morbidity affecting functional mobility. The significant association between use of a walking aid and number of medical conditions in this study supports this hypothesis.

We determined the occurrence of a fall retrospectively. More than half the subjects stated that they had indeed fallen but, because of possible inaccuracies in recollection, this may be a rather conservative figure. Subjects with knee extension strength in the lowest tertile had a significantly greater chance of falling compared with subjects in the highest tertile. Further, our results indicate that performance on TGUG may be used as a screening tool to predict falls in frail older people. Our 15-second threshold is in agreement with others[26], who found that older adults needing ≥ 14 seconds to compete TGUG had an 83% probability of being a faller.

Because sample size was relatively small, some caution is needed when interpreting the importance of non-significant independent variables in our study. However, the fact that muscle strength, habitual physical activity, use of a walking aid, and benzodiazepines were significantly associated with mobility and falling, stresses the

importance of these variables in this patient category. Further, the results were obtained in women. In general, men have more muscle strength reserve capacity and consequently remain longer on the plateau of the strength-function relation[27].

Conclusion

In summary, isometric knee extension strength remains a strong determinant of mobility and was significantly associated with fall occurrence in women referred to a geriatric outpatient clinic. Performing light to moderate household work also remained independently associated with mobility. By assessing strength and activity variables in an outpatient clinic, even with a short test battery, important information is obtained on the risk of mobility impairment and falling in frail older people.

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04

Determinants of vitamin D status in healthy men and women aged 40-80 years

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Abstract

Objectives

To determine the contribution of life style and health related factors on vitamin D status in middle-aged and older men and women.

Study design

A cross-sectional single-centre study in 400 male subjects (40-80 years) and 402 postmenopausal female subjects (56-73 years), conducted in a University Medical Centre in the central part of the Netherlands (52 degrees northern latitude).

Main outcome measures

Medical history, vitamin D, calcium and alcohol intake, physical activity, Body Mass Index, Blood pressure, smoking, total fat body mass and total lean body mass were measured using DEXA. Laboratory analysis included 25-Hydroxyvitamin D (25OHD) and sex hormones.

Results

Thirty six % of men and 51 % of women had 25OHD less than 50 nmol/L. In summertime men had significant higher 25OHD as compared to women (81.5 vs 53.3 nmol/L, P .000) but this difference disappeared come winter. In a saturated model, male gender (B .16, P=.008), and season (summer vs winter B.30, P=.000) remained statistically significant. In men, physical activity and season explained 21% of the variance. In women, household physical activity (B .13, P=.03), sport physical activity (B .02, P=.02) and estradiol (B-.003, P=.048) remained in the model.

Conclusion

In healthy middle-aged and older men and postmenopausal women, male gender and season were important predictors of vitamin D status. In men, physical activity and season, explained 21% of the variance in vitamin D status. In women, physical activity and estradiol explained 9.3% of the variance in vitamin D.

Introduction

Low vitamin D status is commonly described in older people, particularly in nursing homes[1]. Yet, even in healthy, independently living elderly vitamin D status may be compromised. Among European healthy older people (> 70 yrs), 36% of the men and 47% of the women have wintertime serum 25hydroxyvitamin D (25OHD) concentrations < 30 nmol/L[2]. Vitamin D insufficiency is even more common, with two-thirds of community-dwelling elderly women in Northern Europe having a serum 25OHD concentration < 50 nmol/L in winter[3]. These data have prompted the Health Council of the Netherlands to recommend supplementing vitamin D to, amongst other subgroups, women from the age of 50 and men from the age of 70 years[4]. The European Menopause & Andropause Society (EMAS) has recently published a position statement on the role of vitamin D. In healthy postmenopausal women an adequate vitamin D status can be achieved through sun exposure 15 min/day, 3-4 times a week or 800-1000 IU/day vitamin D supplementation[5].

Apart from its well-known function in bone metabolism and calcium homeostasis, evidence is rapidly accumulating on the role of vitamin D and its receptor in many other organ systems, i.e. the immune system, pancreatic/metabolic regulation, the cardiovascular system, muscle and brain function, reproductive function, and control of cell maturation and differentiation affecting the cancer process[6,7].

Many determinants of vitamin D have been reported of which age, season and sun-bathing habits, dietary vitamin D intake, gender and adiposity are important[8,9]. In older people, thinner skin and lower capacity to produce vitamin D, decreased intestinal absorption and decreased hydroxylation in liver and kidneys have also been mentioned as causative factors. In addition, associations between vitamin D and sex hormones both in men and women have been found[10].

In this study we have determined the contribution of gender, age, season, life style factors, hormonal changes and health related factors on vitamin D status in a population of independently living middle-aged and older men and women in the Netherlands (52 degree northern latitude) with an age span of 40 to 80 years .

Materials and Methods

Subjects

This is a cross-sectional, single-center study in 802 independently living men and women 40-80 years of age, in the central part of the Netherlands (52 degrees northern latitude). Male subjects (n= 400, 40-80 years) were extracted from the Hamlet study. Recruitment of subjects (2001-2002) has been published elsewhere[11]. The women (n= 402, 56-73 years) were between 8 and 30 years postmenopausal, and did not use sex steroids after reported last date of menstruation. Recruitment of subjects (1999-2000) has also been published elsewhere[12].

All participants gave written informed consent before enrolment in the study and the institutional review board of the University Medical Centre Utrecht approved the study.

Health-related and life style factors

A medical history was taken. Prevalent diseases were classified using the International Classification of Diseases, 10th revision. Cardiovascular disease (CVD) includes coronary artery disease, peripheral artery disease, and stroke. Chronic disease or medication use was defined as presence of CVD, pulmonary disease, cancer, hypertension, diabetes and/or chronic use of medication.

Physical activity was assessed using the Voorrips questionnaire for the elderly[13], and was divided in household score, leisure score, sport score and an overall score. A lower score means less active. A validated food frequency questionnaire (the Dutch EPIC food frequency questionnaire, EPIC=European Prospective Investigation into Cancer and Nutrition), designed to estimate regular intake of 178 food items in the year prior to enrolment was used to determine alcohol, vitamin D and calcium intake[14]. Smoking was estimated from self-report and categorized in current, former and never smokers. Height and body weight were measured while wearing light indoor clothing, without shoes, and Body mass index (weight in kilograms divided by the square of the height in meters), was calculated. Waist hip ratio was calculated using the waist circumference measured at the level midway between the lower rib margin and the ileac crest with participants in standing position, breathing out gently.

Peripheral blood pressure (BP) was measured twice in the right brachial artery with a semi-automated device (Dynamap). The average of two measurements of systolic and diastolic blood pressure was used for analysis. Total fat body mass and total lean body mass were measured using dual-energy x-ray (DEXA) absorptiometry (Hologic QDR 1000 densitometer, Hologic Inc., Waltham, MA, USA).

Laboratory analyses

Date of blood sampling was recorded to account for seasonal influence. A venapuncture was performed between 08.00 and 10.00 h AM, and fasting blood samples were obtained. 25OHD was determined using an automated assay for the quantitative determination of 25-hydroxyvitamin D (IDS-iSYS, UK). 100% $25OHD_3$ and $25OHD_2$ cospecific. Assay range 13.75-350 nmol/L. Sensitivity 13.75 nmol/L.
Sex hormone binding globulin (SHBG) was measured using an immunometric technique on an Immulite analyzer (Diagnostic Products Corporation, Los Angeles, CA, USA). In the males, testosterone and estradiol were measured using in-house competitive radioimmunoassays after diethyl extraction[11]. In the females serum concentrations of estradiol and total testosterone were measured using radioimmunoassay kits (Diagnostic Systems Laboratories, Webster, TX, USA)[15].

Statistical analyses

The Kolmogorov-Smirnov test and scatter plots were used to test normality. 25OHD was not normally distributed and natural log-transformed for further analyses. Pearson correlation analyses were performed to determine association between ln25OHD and independent life style and health related factors.
To quantify the association between determinants and ln25OHD we used linear regression analysis. Age, lifestyle factors and health-related factors were independent variables in a linear regression model with ln25OHD as dependent variable in the whole group with correction for gender. We repeated the analysis for men and women separately.
Data-analyses were performed using SPSS statistical software (version 17.0; SPSS, Chicago IL, USA). Statistical significance is considered with a P value less than .05, unless otherwise stated.

Results

Patient characteristics are presented in Table 1. Mean 25OHD concentration in men was 62.2 nmol/L as compared to 51.4 nmol/L in women ($P < 0.001$). In men 36% was vitamin D insufficient (25OHD < 50 nmol/L) and vitamin D deficiency (25OHD < 20 nmol/L) was found in 1% , while 31% had a 25OHD concentration $\geq$ 75 nmol/L. In women 51% had a serum 25OHD concentration less than 50 nmol/L. Two percent of women were vitamin D deficient (25OHD < 20 nmol/L) and only 13 % had a 25OHD concentration $\geq$ 75 nmol/L.

In summer (June-August), the difference in 25OHD between men and women was most outspoken (81.5 ± 27.8 nmol/L versus 53.3 ± 18.9 nmol/L, P .000).
In autumn (September-November) the difference declined but remained significant (67.4 ± 23.8 nmol/L versus 48.9 ± 18.2 nmol/L, P .000).
During winter (51.5 ± 20.3 nmol/L vs 51.1 ± 19.0 nmol/L, P .89) and spring (54.9 ± 20.5 nmol/L vs. 53.8 ± 19.4 nmol/L, P .66) no significant difference in vitamin D status was found between men and women.

Individually, gender, BMI (B-.007, P=.04), waist-hip ratio (B -.53, P =.02), dexa total fat mass (B-.43.10-5, P=.03), sport physical activity score (B .02, P=.000), leisure physical activity score (B .005, P=.04), total physical activity score (B .008, P=.00), and season (summer vs. winter B .24, P=.000) were significant determinants of the natural log transformed 25OHD value in the entire study population (Table 2).
In a saturated regression model, male gender (B .16, P=.008), and season (summer vs. winter B.30, P=.000 and fall vs winter B.11, P=.006) remained statistically significant in the model. The explained variance of the model was 12.8%.
In men, sport physical activity score (B .02, P=.006), total physical activity score (B.008, P=.004), smoking (B-.099, P=.05) and season (spring vs winter B-.16, P=.000; summer vs winter B .37, P=.000; autumn vs winter B .12, P=.01) were important determinants of ln25OHD. In a saturated regression model, sport physical activity score (B.014, P=.02), total physical activity score (B. 006, P=.03) and season (summer vs winter B.48, P=.000; autumn vs winter B .29, P=.000) remained significant in the model and the explained variance of the model was 21.1 %.
In women, age (B -.011, P=.03), BMI (B-.011, P=.01), waist hip ratio (B-.60, P=.03), dexa total fat mass (B-.49.10-5, P=.03), systolic blood pressure (B-.002, P=.04), household physical activity score (B .15, P=.008), sport physical activity score (B .021, P=.001), total physical activity score (B.007, P= .007), estradiol (B -.004, P=.004), and season (autumn vs. winter B-.08, P=.05) were significant predictors of ln25OHD. In a saturated regression model, household physical activity score (B .13, P=.03), sport physical activity score (B .02, P=.02), and estradiol (B-.003, P=.048) remained significant in the model, with an explained variance of 9.3%.

Table 1 Characteristics of the study population as a whole and by gender

	Group (n = 802)	Men (n = 400)	Women (n = 402)
Age (years)	63.3 (9.0)	60.2 (11.3)	66.3 (3.9)
BMI (kg/m2)	26.2 (4.0)	26.2 (3.5)	26.2 (4.4)
Waist hip ratio	.89 (0.10)	.97 (0.06)	.81 (.07)
Blood press. syst. (mm Hg)	146 (22)	143 (22)	148 (21)
Blood press. diast (mm Hg)	79 (12)	82 (10)	76 (14)
Dexa total lean mass (kg)	52 (11.5)	62 (7.3)	44 (7.6)
Dexa total fat mass (kg)	20 (8.0)	17 (5.5)	23 (8.9)
Physical activity score			
Total	15.7 (7.6)	18.1 (7.5)	13.3 (7.0)
Household	2.0 (0.54)	1,6 (0.5)	2.3 (0.35)
Sport	2.5 (3.4)	2.5 (3.6)	2.5 (3.3)
Leisure	11.2 (6.9)	13.9 (6.7)	8.5 (6.0)
Chronic disease		194 (48%)	101 (25%)
Vitamin D intake daily (ug)	3.0 (1.3)	3.4 (1.3)	2.6 (1.1)
Calcium intake daily (mg)	1106 (399)	1126 (407)	1085 (388)
25 hydroxyvitamin D (mmol/L)	56.8 (23.0)	62.2 (25.3)	51.4 (18.8)
Alcohol intake (g/day)	14.0 (18.3)	20.2 (21.55)	7.9 (11.32)
Smoking (%)			
Never	35.4	20.5	50.2
Former	45.8	54.3	37.3
Current	18.3	24.3	12.4
Free testosterone (pmol/L)		354.20 (98.10)	.04 (.03)
DHEAS (umol/L)		6.68 (3.29)	13.24 (8.09)
Cortisol (umol/L)		.42 (.14)	.46 (.14)
Estradiol (pmol/L)		91.25 (22.84)	20.19 (13.49)

Data are presented as mean (standard deviation)

Table 2 Linear regression coefficients for the log-transformed 25OHD value

	Ln25OHD Beta N= 802	P-value	Ln25OHD Beta corr. for gender N=802	P-value	Men Beta N=400	P-value	Women Beta N= 402	P-value
Age	-.003	.04	-.5.10-5	.99	.001	.51	-.011	.03
BMI	-.007	.06	-.007	.04	-.002	.75	-.011	.01
Waist hip ratio	.48	.001	-.53	.02	-.42	.26	-.60	.03
Dexa total fat mass	-.80.10-5	.000	-.43.10-5	.03	-.25.10-5	.53	-.49.10-5	.03
Dexa total lean mass	.84.10-5	.000	-.35.10-5	.07	-.5.10-5	.12	.25.10-5	.33
RR syst	-.002	.03	-.001	.10	.000	.68	-.002	.04
RR diast	.002	.10	.000	.74	.001	.71	.001	.45
Diabetes/ hyperglycaemia	-.081	.18	-.063	.29	-.031	.75	-.09	.24
Chronic disease	.07	.02	.03	.39	.042	.33	.006	.88
Cardiovascular disease	.09	.03	.07	.11	.038	.51	.114	.08
Physical activity								
Household score	-.07	.011	.042	.21	-.010	.82	.15	.008
Sport score	.018	.000	.018	.000	.016	.006	.021	.001
Leisure score	.009	.000	.005	.04	.006	.07	.003	.29
Total score	.011	.000	.008	.00	.008	.004	.007	.007
Calcium intake	-1.10-5	.78	-2.2.10-5	.55	-.21.10-4	.69	-2.2.10-5	.65
Vitamin D intake	.038	.001	.017	.15	.015	.37	.02	.24
Totaal testosterone	.01	.000	.006	.10	.006	.13	.02	.55
DHEAS	-.003	.17	.004	.09	.003	.61	.004	.09
Estradiol	.002	.000	-.001	.15	-7.6.10-5	.94	-.004	.004
Cortisol	-.16	.12	-.06	.56	.036	.81	-.155	.26
Alcohol E(10 gr)/dag	.003	.002	.001	.25	.001	.29	.001	.73
Current smoking vs. never	-.04	.36	-.07	.06	-.099	.05	-.027	.65
Season								
spring vs. winter	-.04	.22	-.05	.12	-.161	.000	.072	.09
Summer vs. winter	.26	.000	.24	.000	.368	.000	.053	.37
Autumn vs. winter	-.006	.86	.012	.71	.121	.013	-.08	.05

Data are presented for the total sample, with and without correction for gender, and for men and women separately

Discussion

In our cohort of independently living, middle-aged and older people 36% of men and 51 % of women had an insufficient vitamin D status with a serum 25OHD less than 50 nmol/L, independent of season.
In a multivariable regression model, male gender and season remained significant determinants of vitamin D status in the total sample. In men, season and physical activity were significant determinants after correcting for other variables. In contrast, in women apart from physical activity, estradiol was more important than season in the saturated regression model.

In the last decade, with growing knowledge on the various roles of vitamin D, the cut off points for adequate vitamin D status have gradually gone up, with 25OHD values > 75 nmol/L now considered by some as optimal for vitamin D to exert all its actions[16]. In our sample, a third of men and only 13% of women between 40 and 80 years of age fell in this 'optimal category'.
The lower vitamin D status of women as compared to men has been reported before[17], although in some studies the difference between men and women disappeared in wintertime[18]. The latter is in agreement with our study, although, we have to take into account that men and women were measured in different calendar years. Various explanations are possible for this gender difference. One might be the hormonal changes and reproductive function of women. In 148 pregnant Spanish women serum 25OHD significantly decreased from first to third trimester, independent of season[19]. Another might be that men perform more outdoor activities during work and leisure time, thereby improving their vitamin D status during summer months, as compared to women. However, in both genders, physical activity was an important determinant of vitamin D status.

Men consumed significantly more alcohol than women, and alcohol intake was a significant positive predictor of vitamin D status in the group as a whole. However, after correcting for gender, an effect of alcohol was no longer present. Evidence on alcohol and vitamin D is conflicting. A negative association between alcoholism and vitamin D status, even in subjects with adequate diet, has been reported[20,21]. However, moderate alcohol intake, with an average of about 8 grams/day, was positively associated with 25OHD and bone mineral density[22]. Based on our data, we have no indication of a U-shape relation between alcohol and vitamin D status.

Estradiol was a negative predictor of serum 25OHD concentration in postmenopausal women in our study, even after correcting for other variables. Evidence on vitamin D and estradiol in healthy postmenopausal women is scarce. In 12 postmenopausal women (55-74 yrs), 1 month of estrogen therapy increased total and free calcitriol levels , without changing iPTH levels[23]. In 101 young female volunteers, an inverse association was reported between 25OHD and endogenous estradiol[24].
In addition, evidence from in –vitro studies indicates that estradiol upregulates the vitamin D receptor, activates 1-α hydroxylase, and increases 1,25dihydroxyvitamin D concentration. By promoting the conversion and the function of the active form of vitamin D, higher estradiol is associated with lower serum 25OHD concentration[25-28], which is in agreement with our results.
This was only partly confirmed in a 1-year population based prospective study, in which 72 women were randomly given one of four treatments: hormone replacement therapy (HRT= estradiol and cyproterone acetate), Vitamin D_3+ calcium, HRT + vitamin D_3/calcium, and placebo (calcium). Serum 25OHD increased significantly in the vitamin D_3 and vitamin D_3 + calcium group, but did not change in the other 2 groups, i.e. did not decrease in the HRT group, although serum calcitriol only increased significantly in the HRT group, indicating activation of the active vitamin D metabolite[29].

BMI, waist hip ratio, dexa total fat mass were negatively associated with 25OHD in the population as a whole and in women. This association has also been reported in younger populations. In a prospective study in Colombian schoolchildren between 5 and 12 years of age, vitamin D deficient (25OHD $<$ 50 nmol/L) children had an adjusted 0.8 cm/yr greater change in waist circumference, than did vitamin D sufficient (25OHD $\geq$ 75 nmol/L) children, with a median 30 months follow up period[30].

The relation between vitamin D and obesity seems to work both ways. Vitamin D is fat soluble and is stored in adipose tissue, making it less available to the body[31] and a significant inverse relation was found between 25OHD and subcutaneous and especially visceral adipose tissue[32]. On the other hand, 1α-hydroxylase (the enzyme that activates 25OHD into calcitriol) is expressed in adipose tissue and is functional in cultured adipocytes[33] and calcitriol bound to the vitamin receptor has been found to inhibit adipogenesis[34].

Our study has obvious limitations. Although we accounted for season of measurement, and (outdoor) physical activity, we did not measure sun exposure directly. And because of the cross-sectional design, definitive conclusions on the direction of the relationship between the determinants and vitamin D status are not possible based on this study.

Conclusion

In our cohort of healthy middle-aged and older men and postmenopausal women, male gender and season were important predictors of vitamin D status. In men, remaining physically active and summer season, explained 21% of the variance in vitamin D status. In healthy postmenopausal women, apart from physical activity and season, other variables such as fat mass, and particularly hormonal status play an important role in vitamin D status.

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NOTES

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05

Vitamin D and muscle function

Is there a threshold in the relation?

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Abstract

Objectives

First, to determine the association between serum 25 hydroxyvitamin D (25OHD) concentration and muscle mass, strength and performance. Second, to explore if there is a threshold in the association.

Design: Cross-sectional, single-centre study.
Setting: The central part of the Netherlands (52 degrees northern latitude).
Participants: 802 independently living men and postmenopausal women 40-80 years of age.
Measurements: Health-related and lifestyle factors including physical activity, 25OHD concentration, lean mass, handgrip strength, knee extension strength, and physical performance were determined.

Results

Overall, higher 25OHD level was significantly associated with higher lean mass (22.6 g per nmol/l, 95% CI 7.3;37.9), handgrip strength (0.020 kg per nmol/l, 95% CI 0.001;0.038), and physical performance (0.006 points per nmol/l, 95% CI 0.001;0.012), after adjustment for various confounders. This association was most pronounced below a 25OHD level of 60 nmol/L, with lean mass increase 79.6 g per nmol/L (95%CI 40.8;118.4, $p< 0.01$), handgrip strength 0.09 kg per nmol/L(95%CI 0.045;0.141, $p<0.01$), and physical performance 0.02 points per nmol/L (95% CI 0.005;0.032, $p< 0.01$), and these significant associations attenuated to null above this threshold.

Conclusion

In middle-aged men and (postmenopausal) women, a higher 25OHD level was significantly associated with higher lean mass, muscle strength and performance. These associations were most pronounced below 60 nmol/L and absent above 60 nmol/L, indicating a ceiling effect.

Introduction

In the past decades evidence on the diverse actions of vitamin D has been growing exponentially. In addition to its well-known role in bone metabolism, vitamin D involvement has been reported in auto-immune disease, reproductive function, malignancy, mood disorder, the metabolic syndrome and recently even in sleep disorders[1,2]. One of the major fields of investigation regarding vitamin D has been in the prevention of falls and fractures in the elderly[3-12].

Annually, at least 30 percent of independently living older people experience a fall[13], with a quarter of those who fall having serious injury requiring medical attention and about 6% experiencing a fracture[14]. This has profound implications on quality of life[15] and in a US population based survey no less than 50 % of independently living patients with a fall related injury admitted to hospital were discharged to a nursing home[16].

Several mechanisms have been postulated for a causal role of vitamin D deficiency in falls and fractures. First, vitamin D deficiency may impair bone metabolism and thereby increase proneness to fracture, should a fall occur[17]. Second, vitamin D deficiency may cause muscle weakness[6] and finally it may exert a negative effect on postural stability and body sway[18,19].

In severe vitamin D deficiency, vitamin D supplementation, with or without calcium, did improve muscle function and balance[18,20-22]. However, evidence from meta-analyses on falls and fractures is still inconclusive[23-26]. This may be partly due to the fact that it is unclear what serum hydroxyvitamin D concentration constitutes adequate vitamin D status with regard to bone health and extraskeletal vitamin D actions. International guidelines advise a serum hydroxyvitamin D concentration of 50 nmol/L[27] and 75 nmol/L as adequate[28].

However, in a meta-analysis on fall prevention in older people a minimum serum hydroxyvitamin D concentration of 60 nmol/L was necessary to prevent falls[29], and also dosing interval may play a role in the treatment effect of vitamin D on muscle strength and balance[30].

On the other hand, given the fact that vitamin D is a secosteroid hormone, acting via its well-regulated active metabolite 1α,25 dihydroxyvitamin D on a vitamin D receptor in target organs, it also seems likely that a ceiling effect occurs, meaning that higher serum 25 hydroxyvitamin D concentration will merely act as a reservoir, but will not further improve vitamin D dependent action.

We studied whether serum 25 hydroxyvitamin D is related to muscle mass and function in independently living middle-aged men and women living on 52 degree Northern latitude. Additionally, we explored whether a serum 25OHD concentration of 60 nmol/L constitutes a threshold value in the association between vitamin D and muscle function.

Methods

Subjects

This is a cross-sectional, single-centre study in 802 independently living men and women 40-80 years of age, in the central part of the Netherlands (52 degrees northern latitude). Male subjects (n= 400, 40-80 years) were recruited in 2001-2002 and recruitment of subjects has been published elsewhere[31]. In the men, 11 % had diabetes, 13% cardiovascular disease, 27% hypertension. The women (n= 402, 56-73 years) were between 8 and 30 years postmenopausal, and did not use sex steroids after reported last date of menstruation. Recruitment of subjects (1999-2000) has also been published elsewhere[32]. In the women, 37% had arterial hypertension, 7 % diabetes, 11 % cardiovascular disease.All participants gave written informed consent before enrollment in the study and the institutional review board of the University Medical Centre Utrecht approved the study.

Health-related and life style factors

A medical history was taken, including smoking and alcohol intake. Prevalent diseases were classified using the International Classification of Diseases, 10th revision. Chronic disease or medication use was defined as presence of cardiovascular disease (coronary artery disease, peripheral artery disease and stroke), pulmonary disease, cancer, hypertension, diabetes and/or chronic use of medication.

Physical activity was assessed using the Voorrips questionnaire for the elderly[33], and was divided in household score, leisure score, sport score and an overall score. A lower score means less active.

Height and body weight were measured while wearing light indoor clothing, without shoes, and Body mass index (weight in kilograms divided by the square of the height in meters), was calculated.

Laboratory analyses

A venapuncture was performed between 08.00 and 10.00 h AM, and fasting blood samples were obtained. Serum 25OHD concentrations were determined using an automated assay for the quantitative determination of 25-hydroxyvitamin D (IDS-iSYS, UK), with 100% $25OHD_3$ and $25OHD_2$ cospecificity. Assay range was 13.75-350 nmol/L and sensitivity 13.75 nmol/L.

Handgrip strength

Handgrip strength was measured using an adjustable hand held Dynamometer (JAMAR dynamometer Chicago, IL) at the non-dominant hand. The subjects were seated with their shoulders adducted en neutrally rotated. The elbow was flexed at 90 degrees and the dynamometer was held freely, without support. The forearm was held in a neutral position. The subjects were told to put maximal force on the dynamometer. The average of three attempts (in kg force) was used for analyses.

Knee extension strength

Knee extensior strength was measured using a hand held dynamometer (the Hoggan MicroFET handheld dynamometer, Salt Lake City, UT). The subjects were seated with folded arms on a seat with no arms and a low back. The leg was held in 120 degrees. The participants were instructed to give a maximal push to the dynamometer. Both legs were tested three times and the maximum (in Nm, by multiplying maximum strength and the distance of the dynamometer to the knee joint) was used for analyses.

Physical performance

Physical performance was determined as described by Guralnik et al.[34] and standing balance, walking speed, and ability to rise from a chair, were recorded. Scores were assigned for standing balance from 0-4. For the 8-foot walking test and the repeated chair stand test, those who could not complete the task were given a score of 0. Those completing the test were assigned scores between 1 and 4, based on the quartiles of time needed to complete the test, with the fastest times scoring as 4. Summing up the scores from the chair stand, walking test, and standing balance test created a summary performance score that was used for analyses.

Data analyses

To quantify the association between 25OHD levels and lean mass, muscle strength, and physical performance we used linear regression analysis. Analyses were done for the group, with correction for gender. Confounders, such as age, Body Mass Index, chronic disease, smoking, alcohol and physical activity were first introduced separately, and finally put together in a model. In addition, to determine if a ceiling effect does occur in the association between 25OHD level and lean mass, muscle strength and performance, regression analysis was repeated with 60 nmol/L as cut off point. The association between serum 25OHD level and lean mass, muscle strength and performance was estimated separately above and below 60 nmol/L, again, with adjustment for gender and age, BMI, chronic disease, smoking, alcohol and physical activity. Data-analyses were performed using SPSS statistical software (version 20.0; SPSS, Chicago IL, USA). Statistical significance is considered with a P value less than 0.05.

Results

Patient characteristics including lean mass, muscle strength and physical performance score are presented in Table 1. The average age of the study population was 63.3 (± 9) years, with an average serum hydroxyvitamin D concentration of 56.8 (± 23) nmol/L (range 14 -186 nmol/L). Lean mass increased with 22.6 grams (95% CI 7.3;37.9, $p<0.01$) for each nmol/L increase in 25OHD, after correcting for gender, age, BMI, chronic disease, smoking, alcohol and physical activity, see Table 2.

After adjustment for gender, age, BMI, chronic disease, smoking, alcohol and physical activity, each nmol/L higher 25OHD concentration was associated with a 0.025kg (95% CI 0.006; 0.045, $p=0.01$) increase in handgrip strength and an increase of 0.17 Nm (95% CI 0.01; 0.33, $p=0.04$) in knee extension strength. To determine whether this association between 25OHD and muscle strength was mediated through changes in lean mass, this variable was also included in the model. When also controlling for lean mass as potential intermediate, the association with handgrip strength slightly attenuated to 0.020 kg (95% CI 0.001; 0.038, $p=0.04$) increase per nmol/L 25OHD, while the association between 25OHD and knee extension strength attenuated further and became non-significant (0.10 Nm per nmol/L increase in 25OHD, 95% CI -0.06; 0.26, $p=0.22$) (Table 3).

Table 1 Characteristics of the study population as a whole and by gender

	All (n = 802)	Men (n = 400)	Women (n = 402)
Age (years)	63.3 (9.0)	60.2 (11.3)	66.3 (3.9)
BMI (kg/m2)	26.2 (4.0)	26.2 (3.5)	26.2 (4.4)
Physical activity score			
Total (score)	15.7 (7.6)	18.1 (7.5)	13.3 (7.0)
Household (score)	2.0 (0.54)	1,6 (0.5)	2.3 (0.35)
Sport (score)	2.5 (3.4)	2.5 (3.6)	2.5 (3.3)
Leisure (score)	11.2 (6.9)	13.9 (6.7)	8.5 (6.0)
Chronic disease (yes/no)		194 (48%)	101 (25%)
25 hydroxyvitamin D (nmol/L)	56.8 (23.0)	62.2 (25.3)	51.4 (18.8)
Alcohol intake (g/day)	14.0 (18.3)	20.17(21.55)	7.89(11.32)
Smoking (%)			
Never	35.4	20.5	50.2
Former	45.8	54.3	37.3
Current	18.3	24.3	12.4
Total lean mass (kg)		61.7 (7.3)	44.2 (7.6)
Handgrip strength (kg)		43.3 (8.6)	24.8 (4.8)
Knee extension strength (Nm)		393.8 (79.5)	119.8 (22.0)
Standing Balance score		3.76 (0.59)	3.73 (0.7)
Walking speed score		2.74 (0.83)	2.51 (1.1)
Chair stand score		2.50 (1.12)	2.49 (1.1)
Total Physical Performance score		9.03 (1.8)	8.73 (2.0)

Data are presented as mean (standard deviation)

Each nmol/L increase in 25OHD was associated with a 0.007 point (95% CI 0.002; 0.010, p< 0.01) increase in physical performance score, after correcting for gender, age, BMI, chronic disease, smoking, alcohol, physical activity. To determine if this association between 25OHD and physical performance was mediated through knee extension strength, this variable was also included in the model. This slightly attenuated the effect of 25OHD on physical performance but the association remained

Table 2 **Association between 25OHD and Lean Mass. Regression co-efficients (95% confidence interval)**

	Lean Mass (grams) Beta	P-value
25OHD (nmol/L) corrected for gender	11.8 (-8.1;31.6)	0.25
25OHD (nmol/L) corrected for gender, age, BMI, chronic disease, smoking, alcohol and physical activity	22.6 (7.3;37.9)	< 0.01

significant, i.e. for each nmol/L increase in 25OHD, physical performance improved with 0.006 points (95% CI 0.001; 0.010, p= 0.02) (Table 4).

To determine whether the association between 25OHD on the one hand and lean mass, handgrip strength, knee extension strength, and physical performance on the other hand, experienced a ceiling effect, the association between vitamin D and lean mass, muscle strength, and physical performance was determined separately in a group with 25OHD < 60 nmol/L and ≥ 60 nmol/L (Table 5).

Below 60 nmol/L, there was a strong association between 25OHD and lean mass. For each nmol/L increase in 25OHD, lean mass increased with 79.6 gram (95%CI 40.8;118.4,p < 0.01). In contrast, above 60 nmol/L no association between 25OHD and lean mass could be found.

Below 60 nmol/L, each nmol/L increase in 25OHD resulted in a 0.09 kg (95%CI 0.045;0.141, p<0.01) increase in handgrip strength after controlling for the various confounders, while above 60 nmol/L no significant change in handgrip strength was found with changing 25OHD concentration.

Knee extension strength appeared to be little influenced by 25OHD concentration. Therefore no ceiling effect could be detected.

Below 60 nmol/L each nmol/L increase in 25OHD resulted in 0.02 point (95%CI 0.005;0.032, p<0.01) increase in physical performance. Above 60 nmol/L no significant association between 25OHD concentration and physical performance was found.

Table 3 Association between 25OHD and muscle strength. Regression co-efficients (95% confidence interval)

	Handgrip strength (kg) Beta	P-value	Knee Extension Strength (Nm) Beta	P-value
25OHD (nmil/L) corrected for gender	0.023 (0.002;0.045)	0.03	0.13 (-0.06;0.31)	0.18
25OHD (nmol/L) corrected for gender, age, BMI, chronic disease, smoking, alcohol, physical activity	0.025 (0.006;0.05)	0.01	0.17 (0.007;0.33)	0.04
25OHD (nmol/L) corrected for gender, age, BMI, chronic disease, smoking, alcohol, physical activity and lean mass	0.020 (0.001;0.038)	0.04	0.10 (-0.06;0.26)	0.22

Table 4 Association between 25OHD and physical performance. Regression coefficients (95% confidence interval)

	Physical performance score Beta	P-value
25OHD (nmol/L) corrected for gender	0.01 (0.004;0.016)	< 0.01
25OHD (nmol/L) corrected for gender, age, BMI, chronic disease, smoking, alcohol and physical activity	0.007 (0.002;0.013)	< 0.01
25OHD (nmol/L) corrected for gender, age, BMI, chronic disease, smoking, alcohol, physical activity and knee extension strength	0.006 (0.001;0.012)	0.02

Table 5 **Association between 25OHD and strength, performance and lean mass, below and above 60 nmol/L. Regression co-efficients (95% confidence interval)**

	HYDROXYVITAMIN D	
	< 60 nmol/L*	≥ 60 nmol/L*
Lean Mass (gram)	79.6 (40.8;118.4)	-2.1 (-33.5;29.3)
P- value	< 0.01	0.89
Handgrip strength (kg)	0.09 (0.045;0.141)	-0.02 (-006;0.03)
P- value	< 0.01	0.44
Knee extension strength (Nm)	-0.05 (-0.43;0.33)	-0.18 (-0.55;0.20)
P- value	0.80	0.35
Physical performance (score)	0.02 (0.005;0.032)	0.000 (-0.011;0.012)
P- value	< 0.01	0.96

** Beta, corrected for gender, age, BMI, chronic disease, smoking, alcohol, physical activity*

Discussion

In this study we investigated the association between serum vitamin D levels and lean mass, muscle strength, and physical performance in independently living middle aged men and women in the Netherlands.

We found a significant association between 25OHD and lean mass that persisted after correcting for various confounders. Over the total range of serum 25OHD values, lean mass increased with 22.6 grams for every nmol/L higher 25OHD. However, below 60 nmol/L, the effect of 25OHD on lean mass was much more pronounced; for every nmol/L 25OHD higher lean mass increased with almost 80 grams.

The way in which vitamin D affects muscle function has been thoroughly reviewed recently[35]. Almost 30 years ago, a nuclear vitamin D receptor was first reported in animal and human muscle cells[36,37] en this was confirmed 15 years later[38]. The fact that recently Wang and DeLuca[39] did not find a VDR in skeletal muscle might be explained by the fact that vitamin D has been reported to mediate muscle cell pro-

liferation and differentiation from myoblasts into mature muscle fibres[40], reflecting a variable role of vitamin D in various stages of muscle cell function. Apart from the 'slow' genomic pathway, vitamin D, possibly via a membrane bound receptor[41] rapidly changes intracellular calcium levels[42]. Histologically, muscle fibre atrophy, particularly of type II fibres, has been described in vitamin D deficiency[22,43].

Our study is in agreement with the findings by Visser et al.[44], who found a greater loss of appendicular skeletal muscle mass in a 3 year follow up study in older subjects with low serum 25OHD. In contrast, a sample of community-dwelling adults (age range 21-97), with 42% having serum 25OHD < 50 nmol/L, no consistent association between 25OHD and muscle mass nor strength was found, although lower levels of 1,25OHD were associated with lower skeletal muscle mass in subjects under 65 years of age[45]. Differences in nutritional and especially vitamin D status, comorbidity and physical activity affecting muscle mass as well, might explain these conflicting results.

We found a significant association between 25OHD and lean mass that persisted after correcting for various confounders. Over the total range of serum 25OHD values, lean mass increased with 22.6 grams for every nmol/L higher 25OHD. However, below 60 nmol/L, the association of 25OHD with lean mass was much more pronounced; for every nmol/L higher 25OHD lean mass increased with almost 80 grams. Although we found a significant effect of 25OHD on muscle strength and physical performance below 60 nmol/L, the magnitude of the association was small, much smaller than for lean mass. An explanation is the fact that lean mass indicates the anatomical effect of vitamin D on muscle tissue, while strength and performance are depending on other factors besides 25OHD, such as neurological function, subject and tester motivation.

A small but significant association between 25OHD and handgrip strength, even after correction for gender, age, BMI, chronic disease, smoking alcohol, and physical activity was found and this was also true for knee extension strength, although the association was no longer significant when controlling for lean mass.
When reviewing the available clinical studies on the association between vitamin D and muscle strength, evidence is ambiguous. Correcting vitamin D status in severe vitamin D deficient, symptomatic patients clearly improved muscle strength[20-22], but evidence on objectively quantified muscle strength in older people in relation to

vitamin D status is not abundant. Cross-sectional studies suggest a significant association[46-48] and a lower 25OHD (< 25 nmol/L) concentration increased the risk of loss of handgrip strength and muscle mass in older Dutch subjects[44], although not in moderately to severe disabled older women in Baltimore[49].

Correcting vitamin D status in severe vitamin D deficient (25OHD < 20 nmol/L) frail older women improved muscle strength after 6 months[50]. However, correcting vitamin D status in vitamin D insufficient frail older people, did not improve muscle strength after 6 months in two randomized controlled trials[51,52]. Comorbidity in frail older people that affects muscle strength as well, likely explains the fact that correcting vitamin D insufficiency in this population does not improve muscle strength. Also, compliance with study medication and the achieved serum 25OHD concentration are important factors[53].

At the other end of the health spectrum, in healthy vitamin D replete older volunteers with an average baseline 25OHD concentration of 60-65 nmol/L, improving vitamin D status with either cholecalciferol[54] or 1,25OH2D_3[55] did not improve muscle strength. There appears to be a ceiling effect in the association between vitamin D and muscle strength, which is supported by the findings in our study. Below a serum 25OHD concentration of 60 nmol/L we found a significant positive association between 25OHD and lean mass, handgrip strength, and physical performance, but this association disappeared above 60 nmol/L. Our study confirms the results from Houston et al.[56] in a community sample of men and women between 70-79 yrs of age. As in our study, with the exception of knee extension strength, the slopes of the association between 25OHD and handgrip strength, and physical performance, were significant below a 25OHD threshold, and not significant above this threshold. They found a threshold of 50-69 nmol/L for grip strength and 70-80 nmol/l for physical performance.

In contrast to the fact that muscle strength in vitamin D replete subjects did not improve upon vitamin D supplementation, 36 weeks of supplementation with the active vitamin D metabolite (alfacalcidiol, calcitriol) did reduce the number of fallers in community dwelling vitamin D replete older people[57,58]. This suggests a mechanism separate from muscle function, possibly by improving balance via a vitamin D receptor in neurons. We found a significant association between 25OHD and the Physical performance score, which is the summed score from the standing balance test, walking speed and rising from a chair, particularly below a serum 25OHD concentra-

tion of 60 nmol/L, and even after correction for knee extension strength.
Moreover, the association between 25OHD and knee extension strength was not evident at all in our study, giving the impression that the effect of vitamin D on physical performance is also mediated via other mechanisms such as neuromuscular function. Dhesi et al.[59] found that in vitamin D deficient, older people with a history of falls, vitamin D supplementation improved postural stability and choice reaction time but not muscle strength after 6 months. However, in a more recent double blinded trial in 242 community dwelling older volunteers with serum 25OHD < 78 nmol/L, 1000 mg calcium+ 800IU vitamin D/day for twelve months significantly improved quadriceps strength, body sway by and decreased falls[60].

This study was set out not only to determine the relation between vitamin D and lean mass, muscle strength and performance in middle aged men and women, but also to test the hypothesis that a threshold exists around a serum 25OHD level of 60 nmol/L. This value was based on a meta-analysis on fall prevention[29], indicating that 25OH D levels < 60 nmol/l may not reduce the risk of falling among older individuals, and the supplementation studies[54,55] in healthy vitamin D replete older volunteers with an average baseline 25OHD concentration of 60-65 nmol/L. In the latter studies, improving vitamin D status with either cholecalciferol[54] or 1,25OH2D_3[55] did not improve muscle strength.

Our study has certain limitations. First, because of the cross sectional design definite conclusions on long term consequences cannot be drawn and more longitudinal studies are needed to validate our results. Second, our study population consisted of independently living middle-aged and older volunteers and therefore further research is needed to confirm our results in long term care residents.

Conclusion

In summary, in this study in 802 middle aged men and postmenopausal women, a higher serum 25OHD level was significantly associated with higher lean mass, muscle strength and physical performance. Moreover, this association was most pronounced below a serum 25OHD concentration of 60 nmol/L, and absent above this threshold, indicating a ceiling effect.

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Muscle strength and mobility in vitamin D insufficient female geriatric patients

a randomized controlled trial on vitamin D and calcium supplementation

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Abstract

Background and aims

Insufficient vitamin D status, commonly found in older people, has been associated with muscle weakness. Muscle weakness in old age impairs mobility and is a risk factor for falling. In a randomized, double-blind placebo-controlled trial, we tested the hypothesis that vitamin D + calcium supplementation improves muscle strength and mobility as compared with calcium mono-therapy in vitamin D insufficient female geriatric patients.

Methods

Seventy female geriatric patients > 65 years of age with a serum 25-hydroxyvitamin D_3 (25OHD) concentration between 20 – 50 nmol/L, visiting an outpatient geriatric department, were included. Participants received either cholecalciferol 400 IU/day + calcium 500 mg/day (D/Cal group) or a placebo + calcium 500 mg/day (Plac/Cal group) for 6 months. At baseline and 6 months, muscle strength, power and functional mobility were tested.

Results

At baseline, 25OHD was significantly ($p < .05$) associated with knee extension strength (r .42), handgrip strength (r .28), leg extension power (r .34), Timed Get Up and Go (r -.31) and Modified Cooper test (r .44). At 6 months a significant difference in 25OHD (77.2 versus 41.6 nmol/L, $p < .001$) and 1,25OHD was found between the two groups. Significantly improving vitamin D status in the D/Cal group as compared to Plac/Cal group did not result in a significant difference in strength nor functional mobility between the two groups.

Conclusions

Daily 400 IU vitamin D + 500 mg calcium supplementation is not enough to significantly improve strength nor mobility in vitamin D insufficient female geriatric patients.

Introduction

Older people are prone to develop vitamin D deficiency predominantly because of low sun exposure, decreased capacity of the skin to synthesize pro-vitamin D and inadequate dietary vitamin D intake to compensate[1].

As the serum 25OHD concentration gradually declines from a state of hypovitaminosis D to vitamin D insufficiency (< 50 nmol/L), secondary hyperparathyroidism (sHPT) develops and bone metabolism and muscle function are compromised[2]. Further decline, leading to vitamin D deficiency, is associated with osteomalacia and symptoms of malaise, musculoskeletal pain[3] and muscle weakness, particularly of proximal muscle groups[4]. Histologically, type II muscle fiber atrophy is found in vitamin D deficiency[5].

It is thought that muscle weakness associated with advancing age (sarcopenia) is partly caused by vitamin D deficiency[6]. Indeed, muscle weakness and reduced physical performance have been associated with low vitamin D status in older people[7-9]. However, evidence from randomized controlled trials is conflicting[10,11]. In a meta-analysis done by Latham et al. 13 trials were evaluated based on the used intervention. Of the six trials that studied mono-therapy with vitamin D or an analog, none found a positive effect on muscle strength, physical functioning, nor fall reduction. Combined treatment with calcium and vitamin D showed a reduction in falls in three out of seven trials[11]. This is in agreement with a meta-analysis by Boonen et al. that showed that only combined calcium and vitamin D supplementation reduced hip fracture risk by 18 %[12].

In this paper, we present the results of a randomized, double-blind, placebo-controlled trial on the effect of vitamin D and calcium supplementation as compared with calcium mono-therapy on muscle strength, power and functional mobility in vitamin D insufficient female geriatric patients.

Subjects and methods

Subjects

Women attending the outpatient clinic of the Department of Geriatric Medicine at the University Medical Centre, Utrecht, the Netherlands, were included if they were > 65 years of age, able to walk and follow simple instructions, and had a serum 25OHD concentration between 20 and 50 nmol/l. Exclusion criteria were: treatment with vitamin D or steroids in the previous 6 months, a history of hypercalcaemia or renal stones, liver cirrhosis, serum creatinine > 200 micromol/l, malabsorptive bowel syndrome, primary hyperparathyroidism, uncontrolled thyroid disease, anticonvulsant drug therapy, and presence of any other condition that would likely interfere with the patient's compliance

(i.e. surgery planned). Most women lived in residential homes for the elderly. All subjects gave written informed consent. The study was approved by the Ethics Committee of the University Medical Centre Utrecht, the Netherlands.

Intervention and measurement protocol

Subjects were randomly assigned to either vitamin D (Cholecalciferol) 400 IU/day + calcium 500 mg/day or identically appearing placebo tablets + calcium 500 mg/day. Trial medication was provided by an independent hospital pharmacist who also performed the randomisation. Randomization was done in blocks of six to minimize seasonal influence between the treatment groups. No person involved, i.e. subjects, investigators, nor physicians who treated the subjects, had access to the randomisation procedure. Treatment period was six months (24 weeks $\pm$ 2 weeks). Measurements were done at baseline and 6 months. To stimulate and monitor compliance, subjects were contacted at 3 months to answer questions and repeat information given at baseline. All measurements were done by the principal investigator (first author).

Demographics and anthropometry

Age, medical history, use of medication, body weight while wearing light indoor clothing (to the nearest 0.1 kg), and height (to the nearest 0.1 cm) were recorded. Subsequently, Body Mass Index (kg/m^2) was calculated.

Knee extension strength

Isometric knee extension strength (IKES) was measured with fixed dynamometry. The subject was seated in an adjustable, straight-backed chair with the lower leg unsupported and the hip and knee flexed in a 90° angle, with an adjustable belt around the hips. In both legs, isometric knee extension strength in Newton (N) was measured with a strain gauge applied with a strap around the ankle just proximal to the malleoli[13]. After one 'try out', the best of three measurements was recorded on both sides.

Handgrip strength

Handgrip strength (HGS) was measured using a dynamometer (Takei Kiki Kogyo 5101, Japan). The maximum of three measurements was recorded in kilogram force on both sides[14].

Leg extension power (LEP)

Explosive leg extension power (LEP) was measured with the Nottingham Power Rig[15]. The subject, in a seated position with folded arms and a 90 degree knee angle at the start, pushes a large foot pedal as hard and fast as possible, setting a flywheel in motion. The best of seven measurements was recorded (in Watts, W) on both sides. Between attempts a resting interval of 30 seconds was used.

Timed "Get Up and Go" test (TGUG)

Functional mobility was quantitated with the timed "Get Up and Go" test[16,17]. In this test the time taken by an individual to stand up from a standard arm chair (with a built-in timer), walk a distance of 3 meters, turn, walk back to the chair, and sit down again is recorded. The subject walks through the test once before being timed in order to become familiar with the test. If a participant used a walking-aid in everyday life, this was also used in the test.

Modified Cooper test (COOP)

The Modified Cooper test (COOP)[18], is a test in which the maximum walking distance (in metres) achieved in 2 minutes is recorded. In older people it is used as a measurement for overall mobility. The participant was instructed to walk as fast as she could without starting to run and was taken through the traject before being timed. A walking aid was allowed.

Habitual physical activity

Habitual physical activity was measured with a physical activity questionnaire for the elderly[19]. This is an interview-administered instrument. Activities in three domains (household, sporting, and leisure time) were determined and combined to an overall physical activity score.

Laboratory analyses

Non-fasting blood samples were drawn at baseline and at 6 months to determine 25OHD, 1,25OHD, PTH, calcium, albumin, alkaline phosphatase, phosphate and creatinine.

Statistical analyses

In the Intention to Treat analysis we included all women tested at 6 months. For the Per Protocol analysis subjects were excluded if they met the following criteria: baseline 25OHD $<$ 20 nmol/L or $>$ 50 nmol/L, a compliance of vitamin D/placebo

treatment < 70 %, did not take calcium tablets and a dietary intake of calcium < 500 mg/day, experienced a fracture of the lower extremity during intervention period, or started with corticosteroids during the intervention period.

The Kolmogorov-Smirnov test was used to check normality. Students T, Chi-square and Wilcoxon Signed Ranks test were used to compare the two groups at baseline and 6 months. Analysis of covariance was used to determine between-group effects. In the model, IKES, HGS, LEP, TGUG and COOP were dependent variables with treatment group as a fixed factor and baseline values, age, BMI, MMSE as covariates. Paired samples T-test and Wilcoxon Signed Ranks test were used to determine a within-group difference between 6 months and baseline measurements. Peak values for left and right measurements (IKES, HGS and LEP) were averaged for analysis. Analysis on COOP and TGUG was done using the best attempt. Data is presented as mean with standard deviation, unless stated otherwise. Statistical analysis was done using SPSS statistical package version 11.0.

Results

During a 2 year period ninety-one eligible women were approached (Figure 1). Women who refused were somewhat younger than the study sample (77.8 (± 5.7) yrs). Reasons for refusal were: feeling unable to come to the hospital, a feeling of having enough medication or diagnostic tests already, no interest in research. Seventy women were randomized and started trial medication. Although randomized by an independent hospital pharmacist, the two groups were not completely comparable at baseline. Patient characteristics at baseline were previously presented[20] and are summarized in Table 1. Eleven (15.7%) subjects withdrew from the trial: death (1), cognitive decline (4), a malignant lung tumor (1), recurrent upper urinary tract infections with malaise (2), acute emotional distress (1), hip fracture (1), and peritonitis (1). No adverse events were reported during the intervention period, although three participants reported some nausea when taking the calcium tablets. Compliance to medication, calculated as percentage of trial medication taken, ranged from 59-100% (average 94.8%).

Vitamin D status

At 6 months 25OHD and 1,25OHD significantly improved in the D/Cal group as compared with the Plac/Cal group (respectively 77.2 versus 41.6 nmol/L, p<.001; and 94.1 versus 67.5 pmol/L, p < .001) (Table 2). In the Plac/Cal group 25OHD also improved moderately as compared with baseline (p < 0.05).

Six months intervention significantly reduced PTH with 19 % in the D/Cal group (p .011) and with 17 % in the Plac/Cal group (p .05). No significant difference was found in PTH between the two groups at baseline and at 6 months.

Figure 1 Flow diagram of subjects

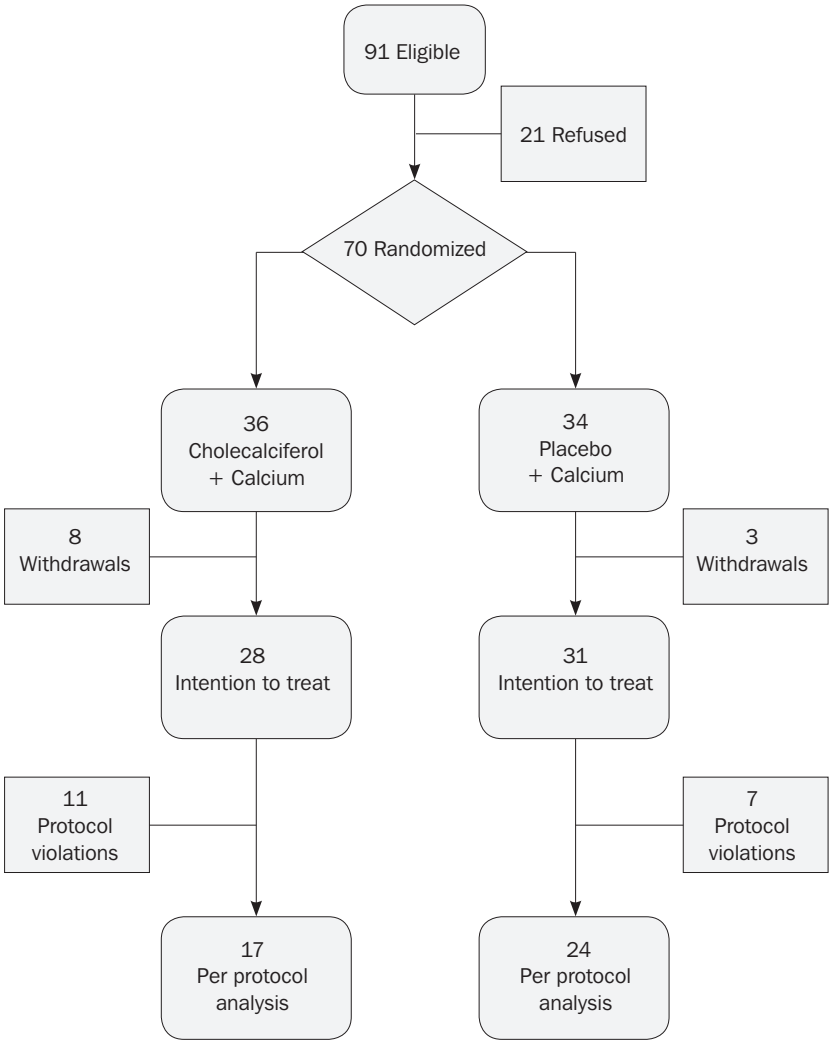

Table 1 Baseline characteristics in the D/Cal group as compared with the Plac/Cal group

	TREATMENT GROUP		
	vitamin D + calcium (n = 36)	placebo + calcium (n = 34)	P-value
Age (yr)	82.4 (6.4)	79.2 (6.7)	.04
Body Mass Index (kg/m2)	26.2 (4.9)	26.7 (4.6)	.68
No. Comorbidity*	2.7 (1.5)	2.1 (1.1)	.06
No. Medication	5.2 (3.4)	4.8 (3.2)	.64

** Comorbidity = diagnosed and/or treated for cardiovascular disease, chronic obstructive pulmonary disease, cerebrovacular disease, dizziness, peripheral neuropathy, lower extremity arthritis, visual impairment, MMSE score < 24, depression*

Table 2 Baseline biochemistry, muscle strength and physical activity and change after 6 months in the D/Cal group as compared with the Plac/Cal group (6 months n = 59)

	TREATMENT GROUP		
	vitamin D + calcium (n = 36)	placebo + calcium (n = 34)	P-value
25OHD (nmol/l)			
Baseline	32.6 (11.6)	34.3 (11.5)	.55
6 Months	77.2 (19.4)	41.6 (19.0)	.00
1,25OHD (pmol/l)			
Baseline	73.0 (22.5)	78.9 (29.0)	.34
6 Months	94.1 (28.9)	67.5 (17.25)	.00
PTH (pmol/l)			
Baseline	10.6 (14.1)	9.1 (5.7)	.55
6 Months	7.1 (4.4)	7.4 (4.4)	.80

Table 2 Continued

	TREATMENT GROUP		
	vitamin D + calcium (n = 36)	placebo + calcium (n = 34)	P-value
Calcium (mmol/l)			
Baseline	2.27 (0.08)	2.30 (0.10)	.31
6 Months	2.29 (0.09)	2.29 (0.13)	.89
Albumin (g/L)			
Baseline	36.9 (3.4)	37.9 (2.5)	.15
6 months	37.3 (2.8)	38.0 (2.8)	.35
Alkaline phosphatase (U/L)			
Baseline	89.3 (22.6)	83.0 (26.9)	.30
6 months	78.0 (21.3)	76.7 (28.4)	.84
Phosphate (mmol/L)			
Baseline	1.13 (.17)	1.07 (.18)	.12
6 months	1.16 (.16	1.09 (.19)	.13
Creatinine (micromol/L)			
Baseline	82.5 (17.1)	80.7 (24.2)	.71
6 months	83.1 (17.0)	85.6 (25.4)	.66
Knee extension strength (N)			
Baseline	172.2 (64.3)	188.6 (60.1)	.29
6 Months	180.2 (76.9)	182.3 (69.6)	.92
Handgrip strength (kg)			
Baseline	15.8 (4.9)	18.0 (4.1)	.04
6 Months	16.7 (5.1)	18.3 (4.4)	.20
Leg extension power (W)			
Baseline	57.2 (30.9)	69.3 (41.0)	.17
6 months	66.6 (30.3)	78.3 (49.6)	.29
Timed Get Up and Go (s)			
Baseline	14.3 (8.2)	12.9 (8.7)	.49
6 months	13.1 (7.0)	12.6 (8.2)	.81
Modified Cooper test (m)			
Baseline	115.0 (39.4)	127.1 (44.2)	.23
6 months	121.7 (40.5)	130.1 (45.2)	.46
Physical activity score			
Baseline	3.0 (2.8)	3.1 (3.1)	.90
6 Months	2.6 (2.2)	2.8 (2.6)	.74

Data is presented as mean (standard deviation)

Muscle strength

At baseline 25OHD was significantly associated with knee extension strength (r. 42, p < .001) and handgrip strength (r .28, p .019), while 1,25OHD and PTH were not. At 6 months no significant relation was found between 25OHD, 1,25OHD, nor PTH and muscle strength. There was no significant difference between the two groups at 6 months in IKES (IKES improved with 2.7 % in the D/Cal group and declined with 2.3% in the Plac/Cal group, p .50) nor HGS (HGS improved 4.8 % in the D/Cal group as compared with 2.1 % in the Plac/Cal group, p .57).
In analysis of variance with knee extension strength and handgrip strength as outcome variables, vitamin D + calcium treatment did not significantly affect muscle strength as compared with placebo + calcium therapy. Analysis in subgroups of strength or vitamin D status, nor a per protocol analysis essentially altered these results (data not shown).

Leg extension power (LEP)

At baseline LEP was significantly correlated with 25OHD (r .34, p .011) and not to 1,25OHD nor PTH. At the 6 month visit the significant correlation between 25OHD and LEP was no longer found (r -.029, p .832). There was no between group difference (D/Cal versus Plac/Cal) in LEP at 6 months in analysis of variance with baseline LEP, age, HGS, MMSE and BMI as covariates.

Timed Get Up and Go test (TGUG) and Modified Cooper test (COOP)

At baseline TGUG and COOP were significantly associated with 25OHD (respectively r -.31 and .44, p < 0.05) and PTH (respectively .37 and - .26, p < 0.05) but not to 1,25OHD. At 6 months the relation between 25OHD and the mobility tests was no longer significant. There was no significant difference between the D/Cal and Plac/Cal group in TGUG nor COOP at 6 months in analysis of variance with baseline values, age, HGS, MMSE and BMI as covariates. No significant difference was found between the 6 month visit and baseline in both groups (data not shown).

Discussion

In this study we wanted to determine the effect of combined vitamin D and calcium therapy on muscle strength, power, and mobility in a sample of female geriatric patients with insufficient vitamin D status. They were mostly situated in homes for the elderly and were referred to the outpatient clinic for analysis of cognitive decline or various physical conditions.

Significantly improving vitamin D status in the D/Cal group as compared to the Plac/Cal group did not result in a significant difference in strength nor functional mobility between the two groups. Moreover, at 6 months the relation between 25OHD and strength and mobility was no longer significant. Vitamin D deficiency is merely one of many conditions that can affect muscle function in older people. In vitamin D deficiency, particularly in the absence of comorbidity, significant improvement in strength and mobility was seen in a matter of weeks upon correcting vitamin D status[4,21]. And even in frail older people, correcting severe vitamin D deficiency led to improved muscle strength[22]. However, correcting a marginally deficient vitamin D status in the presence of comorbidity, as was the case in our sample, appears to have no statistically (and especially no clinically) significant effect on muscle strength nor mobility. This is in agreement with Verrault et al.[23] who found that in frail older women, vitamin D status did not have a relation with future muscle strength nor disability. In addition, Flicker et al.[24] found a significant relation between 25OHD and time to first fall in a nursing home. However, in high level care this relation disappeared, probably due to other conditions causing many falls as well.

In vitro studies have shown a direct effect of vitamin D on muscle fibres through a membrane bound and nuclear vitamin D receptor[25]. Apart from this direct effect of vitamin D on muscle tissue, evidence from in-vivo studies also suggest an effect on neuromuscular tissue. Dhesie and co-workers[26] reported that in a community dwelling vitamin D deficient population, vitamin D supplementation improved postural stability and reaction time, but not muscle strength. This is in agreement with Pfeiffer et al[27], who found a 9 % reduction in body sway after 2 months of vitamin D + calcium therapy in 148 women with 25OHD less than 50 nmol/L. Although we did not find a significant difference between the two groups in the TGUG, a test that combines strength and balance, we cannot exclude a direct neuromuscular effect of vitamin D. Six months of 400 IU vitamin D + 500 mg calcium /day significantly improved both 25OHD and 1,25OHD to within the normal range as previously proposed[2], compared with placebo + calcium therapy. Although our post-intervention serum vitamin D metabolite concentrations are comparable with others that have used higher vitamin D and calcium dosage[28,29], recent meta-analyses have shown that supplementation of calcium and vitamin D in a higher dosage (respectively 1200 mg/d and 800 IU/d) is needed to significantly reduce the risk of hip fractures[12,30]. In our study, PTH declined significantly at six months, but remained at the upper limit of normal, with no significant difference between the two groups. This indicates that a higher 25OHD

concentration than the achieved 77.2 nmol/L in the D/Cal group is needed to fully correct vitamin D status in our sample of female geriatric patients. In a recent study by Adami et al.[31] the need for a much higher serum 25OHD concentration (nearly 120 nmol/L) in elderly individuals with a low calcium intake (median 520 mg/d) in order to maintain serum PTH levels within the normal range, was also reported. Although we used non-fasting bloodsamples that might have influenced serum calcium and PTH values we believe this did not significantly alter our main findings.

We used cholecalciferol (vitamin D_3) to correct vitamin D status. Vitamin D_3 is hydroxylated in the liver and kidneys to become the active vitamin D metabolite, 1α,25OHD, which acts on the vitamin D receptor. We have excluded patients with severe renal and liver dysfunction and found a significant rise in 1α,25OHD in the D/Cal group in contrast to the Plac/Cal group. In contrast, two trials reported a reduction in falls by giving one of the activated D metabolites (i.e. alpha-calcidiol[32] and calcitriol[33]) in vitamin D replete subjects. Up-regulation of the vitamin D receptor might be an explanation, but the precise mechanism is not known.

Our study was a pragmatic trial, because we used vitamin D insufficient subjects with a wide variety of comorbidity, which is both a strength and a limitation. We believe our study has clinical relevance since these are the people that are frequently encountered in everyday geriatric practise, that are prone to fall. Improving strength and mobility by improving vitamin D status would be a safe and inexpensive therapy to reduce the consequences of a fall. The limitation lies in the fact that the "pragmatic approach" resulted in wide standard deviations in strength and mobility results. As was stated by Boonen et al.[12] vitamin D + calcium trials with a positive outcome have generally been conducted in more severe vitamin D deficient populations with excellent compliance. In our opinion, comorbidity, is another important factor in the equation.

Conclusions

Insufficient vitamin D status is frequently encountered in an outpatient geriatric clinic. In our study daily 400 IU vitamin D + 500 mg calcium partially corrected vitamin D status but did not improve muscle strength nor mobility in vitamin D insufficient female geriatric patients with various comorbidity.

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07
General discussion

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Introduction

In the past decades, scientific interest and subsequently the number of publications on vitamin D has grown exponentially. Apart from its classic role in calcium and bone metabolism, vitamin D has been associated with many processes in virtually every organ system[1]. Recently it was suggested that even all sleep disorders are linked to vitamin D, "the hormone that connects us to the sun"[2].

Higher serum 25-hydroxyvitamin D concentration has been associated with reduced overall mortality in some[3], but not all studies[4]. Pooled analysis, from eight randomized controlled vitamin D supplementation trials, in over 70,000 patients (86.8% women, median age 70 years) showed that vitamin D alone does not, but in combination with calcium does reduce mortality (hazard ratio, 0.91; 95% CI, 0.84-0.98). The number needed to treat with vitamin D plus calcium for 3 years to prevent one death was 151[5]. Although many health associations with vitamin D status have been observed, the cause and effect is not always apparent, and an inadequate vitamin D status might just be a marker for poor health, or might even be the consequence of poor health when that leads to reduced outdoor mobility and dietary intake[6]. Before addressing the aims of this thesis a few remarks have to be made on determining what an adequate vitamin D status is.

Determining vitamin D status

Criteria

At a physiological input of vitamin D, whether cutaneous or through diet, there is a rapid conversion of vitamin D to 25-hydroxyvitamin D that is not tightly regulated and practically substrate dependent. In an aggregate study that combined evidence from 1 acute and 5 near-steady state studies, in which vitamin D input was either via oral supplementation (4 studies) or through UV-exposure (2 studies), serum 25-hydroxyvitamin D increased rapidly at low levels of vitamin D, while above a serum vitamin D concentration of 15 nmol/L (corresponding to a daily input of vitamin D of ~2000 IU/day), the hepatic enzyme 25-hydroxylase became saturated and the serum 25-hydroxyvitamin D concentration rose much more slowly with continued supplementation. The start of this slow phase corresponded with a serum 25-hydroxyvitamin D concentration of ~80-100 nmol/L. Thus at a typical input of vitamin D, 25-hydroxyvitamin D is the main circulating vitamin D metabolite, with a half-life of about 3 weeks. It was postulated by the authors that the concentration at which the 25-hydroxyvitamin D production slows down, i.e. when 25-hydroxylase is saturated,

marks the lower end of normal. In this study this was at a serum 25-hydroxyvitamin D concentration of 88 nmol/L[7].
Although 25-hydroxyvitamin D is a stable metabolite, the serum concentration is influenced by liver disease and the use of medication such as anticonvulsants, glucocorticoids, AIDS medication, and antifungals[8].
When the 25-hydroxyvitamin D concentration is low, this leads to a small decrease of 1α,25-dihydroxyvitamin D and calcium absorption. This causes an increase of parathyroid hormone (PTH) secretion, leading to secondary hyperparathyroidism, which in turn stimulates 1α,25-hydroxylase to increase serum 1α,25-dihydroxyvitamin D. PTH has been used as a marker to differentiate between vitamin D insufficiency and sufficiency. However, PTH does not only react to serum 1α,25-dihydroxyvitamin D concentration, but is also influenced by serum calcium and magnesium concentration and by the renal function[9]. Further, immobility, which is often present in older people, causes bone loss, with an increase in serum calcium and subsequent lowering of PTH, irrespective of vitamin D status[10]. Therefore, based on PTH concentration it is difficult to define a cut off point for adequate vitamin D status that is applicable worldwide.

In the past decade, numerous non-skeletal effects of vitamin D have been studied. For most of the non-skeletal conditions such as cardiovascular disease, the metabolic syndrome, and malignant diseases, the available evidence is predominantly assembled from cross-sectional or prospective cohort studies[11]. Based on these studies, it was suggested that higher 25-hydroxyvitamin D concentrations of 75-100 nmol/L are necessary for optimal effect on the endpoints, corresponding with a higher daily vitamin D intake of 1800-4000 IU[12]. However, randomized controlled trials, such as the recently started VITAL trial[13], are necessary to clarify whether higher doses of vitamin D will reduce the risk of cancer and cardiovascular disease.

Another way of determining adequate vitamin D status in an older person is by comparing it to values found in healthy young people in the same population. However, this can lead to an underestimation of vitamin D deficiency, because healthy young people were found to have a low vitamin D status not only in northern Europe, but even in sunny climates. Cultural behaviour towards clothing and sunbathing, and low vitamin D and calcium intake may cause a low serum 25-hydroxyvitamin D concentration even in healthy young people[14-16].

Finally, calcium intake is of influence, not only on PTH concentration, but also on the turnover of 25-hydroxyvitamin D. A low calcium intake leads to a high turnover of 25-hydroxyvitamin D due to the higher production of 1α,25-dihydroxyvitamin D and thus a low calcium intake aggravates vitamin D deficiency[17,18]. Therefore, when determining the adequate level of 25-hydroxyvitamin D, it is also important to consider the average calcium intake in a population.

These different criteria, outcome measures and the significance given to association studies on extra-skeletal effects of vitamin D, fuel the on-going debate among international vitamin D experts regarding the targeted 25-hydroxyvitamin D concentration[19-21]. The United States Institute of Medicine (IOM) determined the Recommended Dietary Allowance (RDA) for vitamin D and calcium in 2011 based on the available evidence on bone health, and regarded evidence on extra-skeletal outcomes of vitamin D as inconsistent at that point. Based on the assumption of minimal sun exposure, a vitamin D intake of 600 IU/d for ages 1-70 years and 800 IU/d for ages > 71 years corresponding to a target 25-hydroxyvitamin D level of 50 nmol/L was advised to cover the needs for 97.5% of the general population. For calcium the RDA for people >50 years of age was set at 1000-1200 mg/d[22].
Taking a different perspective, i.e. an emphasis on patients at risk for vitamin D deficiency and including lower quality, although abundant, evidence on extra-skeletal functions of vitamin D, the Task Force of the Endocrine Society in 2011, advocated a higher target 25-hydroxyvitamin D concentration of 75 nmol/L and a corresponding recommended intake of 1500-2000 IU/d of supplemental vitamin D[23].
The Health Council of the Netherlands updated their dietary reference values for the general population in 2012. In their updated advice they stated that intervention research has proven that vitamin D can reduce the risk of rachitis and fractures, and that it is probable that vitamin D can help protect elderly persons from falling. Effects of vitamin D on other health outcomes were considered inconclusive. For people aged > 70 years supplementation with 20 ug (800 IU) vitamin D/d was advised. For men up to 70 years of age with sufficient sun exposure, no vitamin D supplementation was deemed necessary. For men with insufficient sun exposure (less than 15 to 30 minutes daily exposure to the sun at its highest point, between 11:00 and 15:00 hours, with the head and hands exposed while performing everyday activities) and women between 50-70 years of age 10 ug (400 IU)/d of vitamin D was advised. This intake level corresponds to a target serum 25-hydroxyvitamin D level of 50 nmol/L, which is in line with the population-based advice of the IOM[24].

In vitamin D insufficient women (age range 66-95 years) attending a geriatric outpatient department we found that 400 IU vitamin D + 500 mg calcium per day, improved average serum 25-hydroxyvitamin D from 32.6 (+11.6) nmol/L to 77.2 (+19.4) nmol/L in six months (Chapter 6). In Chapter 4 the average serum 25-hydroxyvitamin D concentration in independently living people between 40-80 years of age was around the target 25-hydroxyvitamin D concentration as advised by the IOM and the Dutch Health Council (56.8 nmol/L + 23 nmol/L, range 14-186 nmol/L). However, 36% of men and 51% of women had a serum 25-hydroxyvitamin D concentration < 50 nmol/L. These percentages and the wide range in serum 25-hydroxyvitamin concentration illustrate on the one hand the large variety in vitamin D status in this population, and on the other hand indicate that a third of the men and half of the women would benefit from supplementation as advised by the Dutch Health Council. **In summary**, based on current evidence, including our own study described in this thesis, the recommended daily supplementation advised by the IOM and the Dutch Health Council seems adequate to achieve a serum 25-hydroxyvitamin D concentration of 50 nmol/L in the majority of people in the Netherlands. The claim that higher serum 25-hydroxyvitamin D levels are needed to fulfil all the extra-skeletal functions has to be confirmed in randomized controlled trials.

25-hydroxyvitamin D assay

To complicate the discussion on adequate vitamin D status and outcome measures further, different assay methods for 25-hydroxyvitamin D are used worldwide and inter-assay variability can be considerable. Two laboratories (one in the Netherlands and one in France) performed a cross-calibration study using three different assays (Competitive protein binding [CPB], Radioimmunoassay [RIA] and Competitive protein binding assay after purification by high-performance liquid chromatography [HPLC]). The mean serum 25-hydroxyvitamin D level in the French laboratory (using CPB) was 80% higher than that measured in the Dutch laboratory (using HPLC) and when using a RIA assay, intermediate values were found. Based on these results a correction factor was determined and after cross-calibration, vitamin D status was actually lower in France, in contrast to the initial findings[25].

In an Australian study in 813 subjects two assay methods (Diasorin Liaison and Liquid Chromatography-Tandem mass Spectrometry, LC-MS/MS) were compared in three different laboratories. The serum 25-hydroxyvitamin D concentrations measured using Diasorin Liaison at one laboratory were on average 26.05 nmol/L lower than those measured using LC-MS/MS at the other laboratory. This difference can have significant

clinical implications when considering patients as vitamin D deficient or not. Using a threshold of 50 nmol/L, based on the Diasorin assay 46% of subjects would be considered deficient, compared with 17% according to the LC-MS/MS assay. And even when using the same technique in two different laboratories (Diasorin Liaison), there was a mean difference of 11.60 nmol/L between the two laboratories. Importantly, despite the differences and misclassification of subjects, correlation coefficients between the assay methods were relatively high ranging from 0.77 to 0.86 indicating the limitations of the correlation coefficient in determining inter-assay agreement[26].
For the studies described in Chapter 4 and 5 we used an automated assay from IDS (IDS-iSYS, Boldon, UK) to determine 25-hydroxyvitamin D. This assay is based on a chemiluminescence method and is 100% 25-hydroxyvitamin D_2 and 25-hydroxyvitamin D_3 co-specific. We conducted a single centre study, using one assay method in all participants, so inter-assay variability between laboratories and different assay methods was not an issue. However, how our 25-hydroxyvitamin D concentrations compare to those from other studies is difficult to assess. An indication is given by the Vitamin D External Quality Assessment Scheme (DEQAS) that is run from the UK and started in 1989. This organisation distributes quarterly 5 samples of unprocessed human serum to about 1200 participants in 54 countries. Laboratories are given approximately 5 weeks to return results. Data are statistically trimmed to produce an All-Laboratory Trimmed Mean (ALTM) and laboratories can compare their results with this ALTM. A recent DEQAS survey on the most used assay methods showed that the IDS-iSYS method was within 4 % of the ALTM[27] (WWW.DEQAS.ORG). This gives an indication of the fairly good accuracy of our assay method, and helps to compare our results with studies using other assay methods.

Apart from the assay method used, several factors affect the accuracy of the 25-hydroxyvitamin D measurement including the variation in collecting tubes and anticoagulants and separating gels used, the possible interference of other lipoproteins with the assay, problems with standardization and a difference in sensitivity for detecting 25-hydroxyvitamin D_2, which is particularly important in the US where supplements contain ergocalciferol (25-hydroxyvitamin D_2). The availability of a Reference Measurement Procedure (RMP), and the development of certified human serum reference materials is important for manufacturers and laboratories to accurately standardise their assay, and for clinicians to compare results from different studies using different assay methods[26].

The aim of this thesis

In this thesis the following topics were addressed:

- The determinants of vitamin D status in middle-aged and older people.
- The relation between vitamin D status and muscle function in middle-aged and older people, and
- The effect of vitamin D supplementation in preserving muscle function and functional mobility in older people.

Determinants of vitamin D status in middle-aged and older people

In contrast to the on-going debate on adequate vitamin D status and the problems concerning assay comparability, there is little controversy around the risk factors for vitamin D deficiency in general. A suboptimal vitamin D status is a worldwide problem and risk factors for a low serum 25-hydroxyvitamin D level are factors that determine vitamin D synthesis in the skin such as clothing, sunbathing behaviour, sunscreen, skin pigmentation, air pollution, latitude, season and time of day. Further, dietary habits, use of supplements and national policies on food fortification are of influence. Finally, female gender and ageing are reported risk factors for a low vitamin D status[28].
In our cohort of independently living men and women between 40 and 80 years of age in the Netherlands, 36% of men and 51% of women had an serum 25-hydroxyvitamin D level less than 50 nmol/L, independent of season (Chapter 4).
Older people, especially in residential care, are particularly at risk of vitamin D deficiency because of less outdoor activities and increased skin coverage when going outside[29]. In addition, skin thickness decreases when people age which leads to reduced capacity of the skin to produce vitamin D[30]. In 433 postmenopausal women, 25-hydroxyvitamin D concentration was positively related to skin thickness[31]. In general, ageing is not associated with significant malabsorption of ingested vitamin D[32] nor does data indicate impaired hydroxylation per se in the elderly, although disturbed liver and renal function or the use of certain medication may impair hydroxylation in older people[33,34].
We found a gender difference in serum 25-hydroxyvitamin level that was most outspoken in the summer, declined in autumn and disappeared in the winter and spring (Chapter 4). Overall, we found that male gender and season were the most important determinants of serum 25-hydroxyvitamin level. In men, season and physical activity were the only significant determinants of vitamin D status. This shows that in healthy

Dutch male volunteers up to an age of 80 years, most of vitamin D is produced in the skin via sun-exposure due to outdoor activities. From October through March, no vitamin D is produced in the skin in the Netherlands, because of the angle of the sun being too low and much UV light being absorbed in the atmosphere[35]. This caused a gradual decline in serum 25-hydroxyvitamin D in men, starting in autumn, to a level comparable to the healthy post-menopausal women in our study, who displayed a rather stable all year serum 25-hydroxyvitamin D average of about 50 nmol/L.

The age-related decline in serum 25-hydroxyvitamin D concentration has been reported to occur earlier in women than in men. In a cross-sectional design in 1107 men and women from the InCHIANTI study, serum 25-hydroxyvitamin D declined with age in both sexes, but earlier in women with a steeper slope starting around 50 years, i.e. in the peri-menopausal period. In men the decline became apparent about 20 years later and was less steep[37]. Postmenopausal thinning of the skin and differences in outdoor activities and sunbathing habits in postmenopausal women as compared to men, can explain the gender difference particularly in the summer 25-hydroxyvitamin D concentration[36]. In addition to differences in the time spent on outdoor activities between men and women, difference in the type and intensity of the physical activity might also explain the gender difference[38].

Further, difference in body composition between men and women might also be an explanation because of an inverse relation between fat mass and serum 25-hydroxyvitamin D concentration[39]. Vitamin D is fat-soluble and in obesity is significantly sequestered in adipose tissue, making it less available to the body[40]. This has also implications when supplementing vitamin D, because intervention studies have shown that increase in serum 25-hydroxyvitamin D upon supplementation is dependent on body mass index[41,42], indicating that obese subjects need a higher vitamin D dosage to achieve a similar 25-hydroxyvitamin D level than non-obese subjects. Given the increase in overweight and obesity prevalence, it can be expected that vitamin D recommended intakes need to be updated in the future.

Finally, susceptibility of vitamin D deficiency is also an inherited trait, with different candidate genes possibly influencing serum 25-hydroxyvitamin D concentration. However, the relative importance of these genes on the serum 25-hydroxyvitamin D concentration has to be determined[43].

In conclusion, vitamin D deficiency is common in older people and particularly women are vulnerable as compared to men, due to reduced vitamin D synthesis in the skin because of less outdoor activities, different clothing and sunbathing habits, higher fat mass and possibly reduced skin thickness around menopause.

The relation between vitamin D and muscle function in older people

Vitamin D and the muscle cell

Almost 30 years ago, a nuclear vitamin D receptor (VDR) was first reported in the muscle cell[44] and since then its role in muscle metabolism and function has become more and more elucidated. However, recently the presence of a VDR in the muscle cell was questioned[45]. By using immunoblotting and immunohistochemical staining, using a sensitive and specific VDR antibody, a VDR could not be detected in mature skeletal muscle tissue. Earlier studies were mostly performed in cultured myoblasts[46-48], and in those studies it was found that vitamin D has a role in muscle cell proliferation and differentiation from myoblasts into mature muscle fibres[49]. This raises the possibility of a varying expression of the VDR in different stages of development of skeletal muscle cells. The role of vitamin D in muscle cells has been thoroughly reviewed recently[50]. Briefly, vitamin D may affect muscle metabolism in four ways:

① By mediating gene transcription via binding to the nuclear VDR and thereby down-regulating proliferation and stimulating differentiation into mature muscle fibres[44,47,49,51].

② By binding to a membrane bound VDR, 1α,25-dihydroxyvitamin D induces rapid changes in the intracellular calcium concentration in the cultured muscle cell, via interacting pathways involving voltage-dependent and calcium-release-activated calcium channels[52-54]. Although 1α,25-dihydroxyvitamin D is generally considered the active vitamin D metabolite, a possible role for 25-hydroxyvitamin D in muscle function was already reported in the mid-seventies[55], suggesting that 25-hydroxyvitamin D influenced phosphate metabolism in diaphragms of vitamin D deficient rats. This effect of 25-hydroxyvitamin D on phosphate metabolism was also found in cultured chick myoblasts[51]. In further support of a role for 25-hydroxyvitamin D in muscle metabolism is the possible binding to an alternative pocket of the VDR and a reported effect on muscle contractile proteins[56].

③ By the allelic variant of the VDR. Different VDR polymorphisms have been reported to affect muscle strength[57-59], although evidence in not yet conclusive and more and larger studies are needed.

④ Indirectly because of a secondary elevated serum PTH and low serum phosphate and calcium concentration, each with its own effect on muscle function[60].

In conclusion, evidence from cultured muscle cells indicates that the vitamin D metabolites can affect muscle function in various ways such as muscle cell differentiation and intracellular calcium handling. Clearly, more research is needed in this

area, to clarify the role of vitamin D in mature muscle fibres, to determine the role of 25-hydroxyvitamin D in muscle function and to confirm the significance of various VDR polymorphisms.

Vitamin D and muscle function in older people

Cross sectional studies in older people indicate a significant association between low vitamin D status and muscle weakness[61-64]. In older Dutch individuals a lower serum 25-hydroxyvitamin D (< 25 nmol/L) increased the risk of loss of muscle mass and handgrip strength after three years as compared to people with serum 25-hydroxyvitamin D > 50 nmol/L[65]. However, a low serum 25-hydroxyvitamin D did not predict disability in moderately-severe disabled older women in Baltimore[66]. Correcting vitamin D status in severe vitamin D deficient (25-hydroxyvitamin D < 20 nmol/L) frail older women improved muscle strength after 6 months, as compared to controls[67].

In Chapter 6, it was shown in a randomized controlled trial in frail older women with a serum 25-hydroxyvitamin D concentration between 20 and 50 nmol/L, that improving this concentration to an average of 77.2 nmol/L (SD 19.4 nmol/L), did not improve handgrip strength, isometric knee extension strength, leg extension power, nor functional mobility after 6 months. However, the group was relatively small and standard deviations were large in the outcome measures. Further, presence of comorbidity, affecting muscle strength and mobility as well, might explain the negative results. At baseline, when the serum 25-hydroxyvitamin D concentration was between 20-50 nmol/L, a significant association between 25-hydroxyvitamin D concentration and handgrip strength, knee extension strength, leg extension power and mobility was found.

At the other end of the health spectrum, muscle loss is also seen in healthy ageing and the possible benefit of supplementing vitamin D on muscle strength in healthy volunteers was investigated. In healthy volunteers with an average baseline serum 25-hydroxyvitamin D level of 60-65 nmol/L, improving vitamin D status did not improve strength[68,69]. In Chapter 5 we tested the hypothesis of a ceiling effect in the relation between 25-hydroxyvitamin D and muscle function and found an indication of a threshold around 60 nmol/L. Below this value a significant positive association was found between 25-hydroxyvitamin D and lean mass, handgrip strength and physical performance. This association disappeared above a serum 25-hydroxyvitamin D level of 60 nmol/L. The presence of a threshold was also re-

ported by Housten et al.[70], with a serum 25-hydroxyvitamin D level of 50-69 nmol/L for handgrip strength and 70-80 nmol/L for physical performance. Thus, negative studies might suffer from a ceiling effect in the relation of vitamin D with muscle function and this is especially important if baseline vitamin D status is not very low. Recently Girgis et al.[50] reviewed the available intervention studies on vitamin D and muscle function. However, comparison is hampered by the heterogeneity of the studies. When looking at the available 17 studies in this review that included middle aged and older people, baseline serum 25-hydroxyvitamin D levels ranged from 15 to 115 nmol/L, while two studies did not report baseline 25-hydroxyvitamin D concentration. Follow up ranged from 16 weeks to 7 years and number of subjects varied from 32 to 33,067. Eight studies showed a positive effect on muscle function, and 9 studies did not report an effect on muscle function. The outcome measures were also diverse with grip strength, lower extremity strength, mobility tests, performance, and balance tests.

When reviewing the available intervention studies on vitamin D supplementation and the risk of falls[50], again heterogeneity in study population, vitamin D dosage, whether or not calcium was given, time of follow up, and inconsistent reporting of fall occurrence hampers comparison. Despite these differences in study design, a recent meta-analysis included 26 trials, with a total of 45,782 participants (78% women and a mean age of 76 years). Vitamin D and calcium combined reduced the risk of falls as compared to placebo (OR 0.83; CI 0.72-0.93), particularly in patients who were vitamin D deficient at baseline[71]. The overall positive effect of vitamin D and calcium supplementation on the risk of falling in vitamin D deficient older people might be explained by the effect on muscle function, although evidence in this area is inconclusive as stated before. Another explanation might be that vitamin D affects balance apart from its effect on muscle function, possibly via a VDR in neurons[72-75].
In summary, based on current evidence including the studies described in this thesis, no definitive conclusions can be drawn regarding the effect of vitamin D supplementation on muscle function in older people. The severity of the vitamin D deficiency, comorbidity and co-medication affecting muscle function as well, compliance with medication and calcium intake are all of influence on the clinical effect of vitamin D on muscle function. Evidence indicates a beneficial effect of vitamin D and calcium supplementation on fall occurrence, particularly in vitamin D deficient older women, possibly by affecting both balance and muscle function.

Concluding remarks and hypotheses

Worldwide, vitamin D deficiency is common in older people and particularly women are vulnerable. In Chapter 4 men displayed a seasonal variation in 25-hydroxyvitamin D, while the healthy postmenopausal women did not seem to benefit from sunlight during the summer. Some evidence indicates that around the menopause, serum 25-hydroxyvitamin D declines more steeply[36]. This decline in serum 25-hydroxyvitamin D is paralleled with a decline in strength that was also found to aggravate around menopause in women[76]. In Chapter 5 an indication of a threshold in the relation between 25-hydroxyvitamin D and lean mass, strength and performance was found. Below a serum 25-hydroxyvitamin D of 60 nmol/L, lean mass, handgrip strength and performance were more affected by the serum 25-hydroxyvitamin D level, than above this threshold. As noted in Chapter 4, even independently living, postmenopausal women have a greater risk of diving below this threshold, at which point vitamin D is likely to significantly affect muscle function.

In addition, this effect on muscle mass and strength has potentially more implications in women as compared to men, because in general men have more strength reserve and are further on the strength-function plateau as compared to women[77-79]. Again, below a certain muscle strength threshold, functional ability becomes affected, with increased risk of falling and subsequently fractures. In Chapter 3, the relation between knee extension strength and functional mobility in seventy vitamin D deficient (25-hydroxyvitamin D 20-50 nmol/L) women attending a geriatric outpatient department was determined. In analysis of variance, knee extension strength and habitual physical activity explained 57% of the variance in the Timed Get Up and Go test and 64% of the 2-minute walking test. This indicates that these women are not on the strength-function plateau and strength is an important determinant of functional mobility in these women.

Finally, in addition to the well-known effects on bone metabolism, and the aforementioned mechanisms regarding muscle function, 25-hydroxyvitamin D level has also been reported to affect balance apart from muscle strength and a positive combined effect of vitamin D and calcium supplementation on fall occurrence was extracted from a large meta-analysis particularly in vitamin D deficient people[71].

Future research

- An important issue that affects all publications on vitamin D is the different 25-hydroxyvitamin D assay methods used, making it difficult to compare and interpret results. The development of certified human serum reference materials and widespread use by manufacturers and laboratories to standardize their results is important.
- Even more relevant than the discussion on what serum 25-hydroxyvitamin D concentration is adequate, i.e. 50 nmol/L or 75-100 nmol/L, is the question how awareness can be raised among general practitioners and physicians caring for older people in nursing homes and hospitals, that vitamin D deficiency is common in older people and supplementation is needed and advised.
- A better clarification is necessary on what vitamin D dosage is needed to achieve a given serum 25-hydroxyvitamin D concentration. This depends of course on average sunlight exposure, but also on average calcium intake in a given country. The role of morbid obesity, another world wide problem, has to be taken into account in future recommendations. The role and implications of a genetic susceptibility to vitamin D deficiency also needs further research.
- More research is needed on the presence of the VDR in muscle cells and the possibility of 25-hydroxyvitamin D binding to an alternative pocket of this receptor.
- Randomized controlled trials are necessary to clarify what is indicated in abundant cross-sectional studies on the relation between vitamin D and various extra-skeletal functions.
- Large randomized controlled trials in older people on vitamin D supplementation and muscle strength, determined with quantitative muscle strength measurements, and measuring baseline and post-intervention serum 25-hydroxyvitamin D status with standardised assays, would be needed to settle the discussion on the usefulness of vitamin D supplementation in order to improve muscle function in older people. However, given the current Dutch recommendations on vitamin D supplementation in older people, placebo-controlled trials are no longer possible in the Netherlands, apart from determining the effect of a higher vitamin D intake on muscle strength as compared to the currently recommended intake.

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08

Summary
Samenvatting

Summary

Vitamin D deficiency is a worldwide problem particularly among older people, who are more susceptible to a low vitamin D status. Less sun exposure and reduced skin thickness with advancing age result in a diminished vitamin D synthesis in the skin. In addition, dietary intake of vitamin D is too low to compensate for the reduced synthesis in the skin. Apart from its well-known role in bone metabolism, vitamin D has been implicated in a myriad of (patho)physiological functions in virtually every organ system, including muscle function. Determining the effect of vitamin D on muscle function in older people is especially important because of the possible consequences for mobility, maintaining an independent lifestyle later in life and for the occurrence of falls and fractures.

Research in this thesis has been performed in two populations. The determinants of vitamin D status were studied in independently living middle-aged and older men and postmenopausal women (40-80 yrs of age) living in the Netherlands (52 degrees northern latitude). Also, the association between the vitamin D status and muscle function was determined in this group. In addition, in frail older women with a low vitamin D status (serum 25-hydroxyvitamin D level 20 – 50 nmol/L), the effect of vitamin D + calcium supplementation on muscle function and functional mobility was assessed. Finally, determinants of functional mobility in these frail women were determined.

In **Chapter 1** a general introduction is given on vitamin D metabolism and on sarcopenia, the age-related loss of muscle mass and strength.

In **Chapter 2** evidence on vitamin D deficiency, muscle function and falls in older people was reviewed, based on in-vitro research in cultured muscle cells, case reports, cross-sectional and intervention studies in older people. From the available evidence it was concluded that vitamin D deficiency can affect muscle function in older people, particularly if serum 25-hydroxyvitamin D is very low. However, the most vulnerable people to have a low vitamin D status are the frail, homebound older people and in this population comorbidity affects muscle function and mobility as well. In contrast, loss of muscle mass and strength is observed even in healthy older people, and vitamin D supplementation did not prevent this. In the remaining chapters the determinants of vitamin D status and the nature of the relation between vitamin D and muscle function is explored.

The most important contributors of functional mobility in frail vitamin D insufficient (25-hydroxyvitamin D 20 – 50 nmol/L) older women are described in **Chapter 3**. In analysis of variance, knee extension strength, habitual physical activity and use of a walking aid were significantly related to performance on mobility tests. Fifty-three percent of the women had experienced a fall in the previous 6 months. Low knee extension strength, worse performance on the mobility tests, use of benzodiazepines and use of a walking aid were associated with fall occurrence.

Determinants of vitamin D status in independently living middle-aged and older men and postmenopausal women (40 – 80 yrs of age) are described in **Chapter 4.** Thirty-six percent of men and 51% of the women had a serum 25-hydroxyvitamin D concentration less than 50 nmol/L. Overall, male gender and season were the most important determinants of serum 25-hydroxyvitamin D concentration. This gender difference in serum 25-hydroxyvitamin level was most outspoken in the summer, declined in autumn and disappeared in the winter and spring. In men, season and physical activity were the only significant determinants of vitamin D status, which shows that in healthy Dutch male volunteers up to an age of 80 years, most of vitamin D is produced in the skin via sun-exposure due to outdoor activities. In contrast, in the independently living post-menopausal women a rather stable all year serum 25-hydroxyvitamin D average of about 50 nmol/L was found. In these women, household and sport physical activity were positively associated with serum 25-hydroxyvitamin D and estradiol negatively. The observed effect of estradiol might be the result of up-regulation of the VDR and activation of 1α-hydroxylase, resulting in a higher turnover of 25-hydroxyvitamin D, and increase of the 1α,25-dihydroxyvitamin D concentration.

In **Chapter 5** the association between serum 25-hydroxyvitamin D concentration and muscle function was assessed in the same sample of 802 men and postmenopausal women between 40 and 80 yrs of age. There was a significant association between serum 25-hydroxyvitamin D and lean mass that was most pronounced below a 25-hydroxyvitamin D level of 60 nmol/L, and absent above this threshold. For each nmol/L 25-hydroxyvitamin D higher, lean mass increased by almost 80 grams, after correcting for various confounders. We also found a significant association between 25-hydroxyvitamin D and handgrip strength and physical performance below 60 nmol/L, but the magnitude of the effect was smaller as compared with lean mass. The results in this study indicate that there may be a ceiling effect in the relation between 25-hydroxyvitamin D and muscle function.

Chapter 6 reports on the results of a randomized controlled trial in seventy frail older women, with a serum 25-hydroxyvitamin D concentration between 20 and 50 nmol/L. Six months of 400 IU/d cholecalciferol + 500 mg/d calcium supplementation significantly improved 25-hydroxyvitamin D concentration as compared with calcium mono-therapy (77.2 vs 41.6 nmol/L respectively). At baseline, when serum 25-hydroxyvitamin D was between 20 and 50 nmol/L, a significant association between 25-hydroxyvitamin D and handgrip strength, knee extension strength, leg extension power and mobility was found. However, improving serum 25-hydroxyvitamin D concentration did not improve handgrip strength, isometric knee extension strength, leg extension power, nor functional mobility as compared with calcium mono-therapy after 6 months. However, the group was relatively small and standard deviations in the outcome measures were large. Further, presence of comorbidity, affecting muscle strength and mobility as well, might explain the negative results.

Various criteria that are used to determine what constitutes an adequate vitamin D status in older people, are discussed in **Chapter 7**. The significance that is given to studies in which vitamin D is associated with various extra-skeletal functions such as cardiovascular and auto-immune disease, and malignancy, results in different (inter)national guidelines regarding vitamin D supplementation. Further, different assay methods used to determine serum 25-hydroxyvitamin D concentration complicate comparison between studies and possible solutions for the near future are addressed. The role of vitamin D in muscle function in older people is discussed, based on the available evidence thus far, including the studies described in this thesis. Concluding remarks, remaining questions, and suggestions for future research complete the general discussion.

Samenvatting

Vitamine D-deficiëntie is een wereldwijd probleem en met name oudere mensen hebben een hoger risico op een tekort aan vitamine D. Diverse factoren zoals minder zonlichtexpositie en een dunnere huid naarmate mensen ouder worden, zorgen ervoor dat er minder vitamine D in de huid wordt geproduceerd. Bovendien is de inname van vitamine D via de voeding in het algemeen te gering om de verminderde productie in de huid te compenseren. Vitamine D wordt, naast een klassieke rol in regulering van het bot metabolisme, geassocieerd met diverse (patho)fysiologische functies in vrijwel elk orgaan systeem, waaronder ook de spierfunctie. Het is van belang te bepalen wat het effect van vitamine D op spierfunctie bij oudere mensen is vanwege de mogelijke gevolgen voor mobiliteit, behoud van een onafhankelijke levensstijl en het voorkomen van valincidenten en fracturen.
De gegevens in dit proefschrift zijn afkomstig van studies in twee populaties. In zelfstandig wonende Nederlandse mannen en postmenopauzale vrouwen tussen de 40 en 80 jaar is onderzocht wat de determinanten van de vitamine D status zijn. Vervolgens werd in deze groep vastgesteld wat de relatie is tussen de vitamine D status en de spierfunctie. In fragiele oude vrouwen met een lage vitamine D-status (serum 25-hydroxyvitamin D niveau 20 – 50 nmol/L) werd onderzocht wat het effect is van vitamine D + calcium suppletie op spierfunctie en functionele mobiliteit. Ten slotte werden in deze groep fragiele vrouwen de determinanten van de functionele mobiliteit vastgesteld.

In **Hoofdstuk 1** wordt een algemene inleiding gegeven over het vitamine D metabolisme en over sarcopenie, het verlies van spiermassa en kracht naarmate mensen ouder worden.

In **Hoofdstuk 2** wordt een literatuuroverzicht gegeven van de rol van vitamine D in spierfunctie, spierkracht en het voorkomen van valincidenten, gebaseerd op laboratoriumonderzoek in spiercellen, case-reports, cross-sectionele en interventie studies in ouderen. Uit de beschikbare gegevens werd geconcludeerd dat vitamine D deficiëntie invloed kan hebben op spierfunctie in oudere mensen, met name als de serum 25-hydroxyvitamin D concentratie zeer laag is. Echter, een dergelijke vitamine D-status komt met name voor bij fragiele, minder mobiele mensen, waarbij comorbiditeit en co-medicatie ook van invloed zijn op spierfunctie en mobiliteit. Bovendien wordt verlies van spiermassa en kracht ook beschreven in gezonde ouderen en uit de beschikbare studies blijkt dat dit niet kan worden voorkomen door vitamine D-suppletie. In de volgende hoofdstukken van dit proefschrift worden de determinanten

van de vitamine D status en de aard van de relatie tussen vitamine D en spierfunctie in ouderen verder onderzocht.

In **Hoofdstuk 3** wordt beschreven wat de belangrijkste determinanten van functionele mobiliteit zijn in fragiele oudere vrouwen met een lage vitamine D status (25-hydroxyvitamin D 20 – 50 nmol/L). In variantieanalyse bleek dat kniestrekkracht, lichamelijke activiteit en het gebruik van een loophulpmiddel significant geassocieerd waren met functionele mobiliteit. Drie-en-vijftig procent van de vrouwen was een keer gevallen in de voorafgaande 6 maanden. Lage kniestrekkracht, verminderde mobiliteit, gebruik van benzodiazepinen en gebruik van een loophulpmiddel waren geassocieerd met het optreden van een val.

In **Hoofdstuk 4** worden determinanten van vitamine D-status in zelfstandig wonende mannen en postmenopauzale vrouwen, met een leeftijd tussen de 40 en 80 jaar, beschreven. Zesendertig procent van de mannen en 51% van de vrouwen had een serum 25-hydroxyvitamine D concentratie beneden de 50 nmol/L. In de gehele groep bleek dat mannelijke geslacht en seizoen de belangrijkste determinanten waren van de serum 25-hydroxyvitamine D concentratie. Dit verschil tussen mannen en vrouwen in serum 25-hydroxyvitamine concentratie was het meest uitgesproken in de zomer, daalde in herfst en verdween in de winter en het voorjaar. In de mannen waren seizoen en fysieke activiteit de enige significante determinanten van vitamine D-status. Hieruit blijkt dat in gezonde Nederlandse mannen tot een leeftijd van 80 jaar, vitamine D status met name wordt bepaald door vitamine D productie in de huid als gevolg van activiteiten buitenshuis. Dit in tegenstelling tot de bevindingen in zelfstandig wonende postmenopauzale vrouwen, waarbij een tamelijk stabiel serum 25-hydroxyvitamin D gemiddelde van ongeveer 50 nmol/L werd gevonden, onafhankelijk van de seizoenen. In deze vrouwen bleek lichamelijke activiteit in het huishouden en sport positief geassocieerd met de serum 25-hydroxyvitamine D concentratie. De estradiol concentratie was negatief geassocieerd met de serum 25-hydroxyvitamine D concentratie. Dit negatieve effect van estradiol kan verklaard worden door gegevens uit in-vitro studies waarbij estradiol de vitamine D receptor expressie stimuleerde en 1α-hydroxylase activeerde, wat resulteert in een hogere 'turn-over' van 25-hydroxyvitamine D, en een verhoging van de 1α,25-dihydroxyvitamine D concentratie.

In **hoofdstuk 5** wordt de associatie tussen de serum 25-hydroxyvitamine D concentratie en spierfunctie beschreven in 802 mannen en postmenopauzale vrouwen tussen de

40 en 80 jaar oud. Er was een significante associatie tussen de serum 25-hydroxyvitamine D concentratie en de vetvrije massa en deze associatie was het meest uitgesproken beneden een 25-hydroxyvitamine D niveau van 60 nmol/L en afwezig boven deze drempel. Voor elke nmol/L 25-hydroxyvitamine D hoger, steeg de vetvrije massa met bijna 80 gram, na correctie voor diverse beïnvloedende variabelen. Er werd ook een significante associatie tussen de 25-hydroxyvitamine D concentratie en handgreepkracht en functionele mobiliteit gevonden onder de 60 nmol/L, maar het effect was kleiner dan bij de vetvrije massa. De resultaten in deze studie suggereren dat er sprake is van een 'plafond effect' in de relatie tussen 25-hydroxyvitamine D en spierfunctie.

In **Hoofdstuk 6** worden de resultaten gepresenteerd van een gerandomiseerde, placebo-gecontroleerde trial in 70 fragiele oudere vrouwen, met een serum 25-hydroxyvitamine D concentratie tussen 20 en 50 nmol/L. Zes maanden suppletie met 400 IU/d cholecalciferol + 500 mg/d calcium verbeterde de serum 25-hydroxyvitamin D concentratie significant ten opzichte van de calcium monotherapie (respectievelijk 77.2 nmol/L versus 41.6 nmol/L). Voor de start van de interventie, toen de hele groep een serum 25-hydroxyvitamine D concentratie tussen 20 en 50 nmol/L had, werd een significante associatie gevonden tussen 25-hydroxyvitamine D concentratie en handgreepkracht, kniestrekkracht, 'spierpower' en mobiliteit. Echter, verbetering van de serum 25-hydroxyvitamine D concentratie door 6 maanden suppletie leverde geen significante verbetering op in handgreepkracht, kniestrekkracht, 'spierpower' noch functionele mobiliteit ten opzichte van calcium monotherapie. Echter, de groep was relatief klein en de standaarddeviaties in de uitkomstparameters waren groot. Daarnaast kan de aanwezige comorbiditeit eveneens van invloed zijn geweest op de spierkracht en mobiliteit.
In **Hoofdstuk 7** worden de voor-en nadelen van de diverse criteria, om een adequate vitamine D status in ouderen te bepalen, besproken. De waarde die gehecht wordt aan studies waarin vitamine D geassocieerd wordt met diverse aandoeningen, zoals auto-immuunziekten, hart-en-vaatziekten en maligniteiten, leidt tot verschillen in internationale richtlijnen ten aanzien van vitamine D suppletie. De problemen die kunnen ontstaan vanwege het feit dat er diverse laboratoriummethoden beschikbaar zijn voor het bepalen van de serum 25-hydroxyvitamine D concentratie worden eveneens aangestipt. Op basis van de beschikbare gegevens tot nu toe, inclusief de studies die worden beschreven in dit proefschrift, wordt de rol van vitamine D in de spierfunctie bij ouderen bediscussieerd. Openstaande vragen en suggesties voor verder onderzoek completeren de discussie.

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Appendix

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Curriculum Vitae

Hennie Janssen werd geboren op 9 april 1971 te Budel. Na behalen van het VWO diploma in 1989 aan het Bisschoppelijk College te Weert, studeerde zij Medisch Beeldvormende en Radiotherapeutische Technieken (MBRT) van 1989 tot 1993 aan de Hogeschool Eindhoven. Nadien studeerde zij geneeskunde aan de Universiteit Maastricht. Tijdens haar studie deed ze onderzoek naar de neurale buis sluiting bij embryo's onder begeleiding van dr. H.W.M. van Straaten in Maastricht en naar de rol van de cervixlengte in het ontstaan van prematuriteit onder begeleiding van dr. K. Dijkstra in New York.

Na het arts examen (cum laude), startte ze in november 1999 met het in dit proefschrift beschreven onderzoek in het Laboratorium Mobiliteit van de afdeling Geriatrie van het UMC Utrecht, aanvankelijk onder begeleiding van prof. S.A. Duursma, dr. H.J.J. Verhaar en dr. M.M. Samson en later onder begeleiding van prof. Y.T. van der Schouw, dr. M.H. Emmelot-Vonk en dr. H.J.J. Verhaar.

In januari 2003 startte ze met haar interne vooropleiding in het Jeroen Bosch ziekenhuis te 's-Hertogenbosch, in eerste instantie in het kader van de opleiding tot klinisch geriater, maar vanaf 2005 in het kader van de opleiding longziekten en tuberculose. Sinds 2010 is ze werkzaam als longarts/slaapgeneeskundige in Kempenhaeghe, een derde lijns expertisecentrum voor slaapgeneeskunde te Heeze.

NOTES