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Efficacy of Vitamin D3 Supplementation on Physical Performance in Community-Dwelling Older Adults

A Randomized Parallel Double-Blinded Placebo-Controlled Pilot Study

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Master Thesis | Translational Medicine

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ABSTRACT: ***Background***: Impaired lower extremity muscular strength and mobility are major risk factors for falls in elderly. Vitamin D insufficiency has as well been associated with increased risk of falls and impaired physical performance. In addition, vitamin D receptors (VDR) have been identified in muscle fibers, suggesting that vitamin D has a direct effect on muscle tissue. These effects are thought to be mediated through genomic and non-genomic pathways and also through inhibition of muscle inflammation, which is often observed in elderly. ***Methods***: The present randomized parallel double-blinded placebo-controlled pilot study was proposed to investigate the effect of a daily dose of 50 µg (2000 IU) vitamin D_3 (n=0) or placebo (n=5) on physical performance parameters in Danish elderly (72.8 ± 5.5 years) at baseline and after 12 weeks of treatment. Primary outcomes were maximal muscle strength (MVC) and Timed Up and Go (TUG) Test. Secondary outcomes, in this study, were pressure pain threshold (PPT), Berg Balance Scale , EQ-5D-5L score, Falls Efficacy Scale-International score, fall diaries and 25(OH)D serum levels. ***Results***: All subjects were allocated placebo treatment, why comparisons of treatment groups were not possible. Paired t-tests for the two time points showed significant differences for MVC (p = 0.01) and TUG (p = 0.05). For secondary outcomes, only 25(OH)D serum levels were found to be significantly increased post-treatment (p = 0.02). Furthermore, correlations between outcomes and 25(OH)D levels were examined, revealing that 25(OH)D serum levels correlated positively with PPT post-treatment (test point 1 (TP1) r = 0.913, p = 0.031 and test point 2 (TP2)) r = 0.964, p = 0.008). ***Conclusion***: In the present study it was not possible to conclude whether a daily dose of 50 µg vitamin D_3 is able to improve muscle strength or time to complete the TUG test in elderly after 12 weeks of treatment.

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1 Introduction

Each year approximately 30 % of elderly above the age of 65 years will experience a fall, and the risk increases with older age (1). Approximately 20 % of these falls require medical attention and 5 % is going to result in fractures or other fall-related injuries (1). The Danish emergency rooms annually treat approximately 40.000 elderly above 65 years in relation to a fall (2). Falls are resulting in increased morbidity and mortality for those involved (3) and therefore lead to increased use of health care services. The number of falls and related expenses will continue to increase due to the increasing number of senior citizens in our society (4). Therefore, falls position as a very important health problem. Risk factors for falling include older age, counting decreased muscle strength, decreased balance, gait deficits and visual impairment together with a past history of falling (5,6). Vitamin D deficiency is a worldwide problem, but is especially pronounced at north-

ern latitudes where sun exposure during winter time is limited. Approximately 80 % of non-institutionalized Danish adults above the age of 65 suffers from vitamin D insufficiency, i.e. a 25(OH)D serum level < 50 nmol/L, while 44 % of institutionalized Danish elderly suffers from severe vitamin D deficiency, i.e. a 25(OH)D serum level < 12,5 nmol/L (7,8). Vitamin D deficiency is characterized as a 25(OH)D serum level < 25 nmol/L (7,8). Low levels of 25(OH)D have been associated with decreased muscle strength and balance, falls and an increased risk of fractures (9-12). Sufficient amounts of vitamin D are rarely obtained through the normal Danish diet alone (13). At Danish latitudes, sunlight is accordingly an important source of vitamin D. People with adequate exposure to sunlight may therefore have no need of vitamin D in their diet (14), however, since sunlight is limited during the Danish winter, supplements at this time of year are especially beneficial. The increased risk of vitamin D deficiency in the elderly may be due to factors such as reduced dietary intake, decreased exposure to sunlight, poor absorption through intestines and decreased conversion in the liver and kidney (15-18).

This report will in the following review the literature on the metabolism of vitamin D, its effect on musles, and the changes that are observed in elderly due to aging. In addition, a randomized double-blinded placebo-controlled pilot study was conducted in order to investigate the effect of vitamin D_3 on muscle strength and functional mobility in this population.

1.1 Vitamin D Metabolism

Vitamin D is a lipophilic secosteroid and it is well-known for its actions on calcium and phosphate metabolism and bone health (19,20). Vitamin D_3 is the most important source of vitamin D and is formed in the epidermis by irradiation of 7-dehydrocholesterol to previtamin D_3 which is further converted into vitamin D_3 by a temperature-dependent reaction (21-23). 7-dehydrocholesterol is a compound normally found in the skin, which has also been found to be decreased with age (24). An overproduction of vitamin D_3 in case of overexposure to sunlight is prevented as excess amounts of vitamin D_3 are converted into inactive compounds (25). In addition to the epidermic production, vitamin D can be obtained from dietary sources in the forms of vitamin D_3 present e.g. in oily fish, and vitamin D_2 which is found in mushrooms (14,25-28). It is assumed that vitamin D_2 is metabolized similarly to vitamin D_3 and they are believed not to differ in their mechanism of action (29). However, it has been discussed whether the two metabolites are bioequivalent (30), and *Heaney et al., 2011* showed that vitamin D_3 is more potent than vitamin D_2 in ability to raise and maintain 25(OH)D serum levels (31).

After formation in the skin or absorption in the intestines, vitamin D_3 is transported to the liver by vitamin D-binding protein (VDBP), which is

the major transport protein of the vitamin D metabolites in the blood (32,33). The first step of activation of vitamin D_3 occurs in the liver where it is hydroxylated into 25-hydroxyvitamin D_3 ($25(OH)D_3$) by 25-hydroxylase. (22,34,35) This process is confined since $25(OH)D_3$ regulates the rate of 25-hydroxylation and, thus, the levels of $25(OH)D_3$ are regulated by a negative feedback mechanism which is very important for two reasons(25): Firstly, the feedback mechanism thereby accurately regulates the concentration of $25(OH)D_3$ in the plasma which prevents excessive action of vitamin D_3, when the intake or production of vitamin D_3 fluctuate in a wide range (25). Secondly, once vitamin D_3 has been converted into $25(OH)D_3$, it has a half-life of two weeks and it will not be stored opposite to vitamin D_3 which can be stored in the liver for months (25,36). $25(OH)D_3$ is transported in the plasma to the kidneys where it is further hydroxylated in the renal tubules into the biological active metabolite 1,25-dihydroxyvitamin D_3 ($1,25(OH)_2D_3$) (22,25,32). The above described processes are illustrated in *figure 1*. Although the 1α-hydroxylase enzyme, responsible for the conversion of $25(OH)D_3$, is primarily found in the proximal renal tubular epithelial cell, the enzyme is also found locally in extrarenal sites, such as immune cells and muscle cells (37-40). The renal production of $1,25(OH)_2D_3$ is tightly regulated by the parathyroid hormone (PTH), which stimulates 1α-hydroxylase, and by serum calcium and phosphorus levels (25,38). $1,25(OH)_2D_3$ has a rapid clearance, compared to $25(OH)D_3$, and a half-life of only 4-6 hours (41). For vitamin D_2 both of the above described metabolites are named $25(OH)D_2$ and $1,25(OH)_2D_2$, respectively. In the following vitamin D, 25(OH)D and $1,25(OH)_2D$ will be used to describe vitamin D_2 and D_3 metabolites collectively.

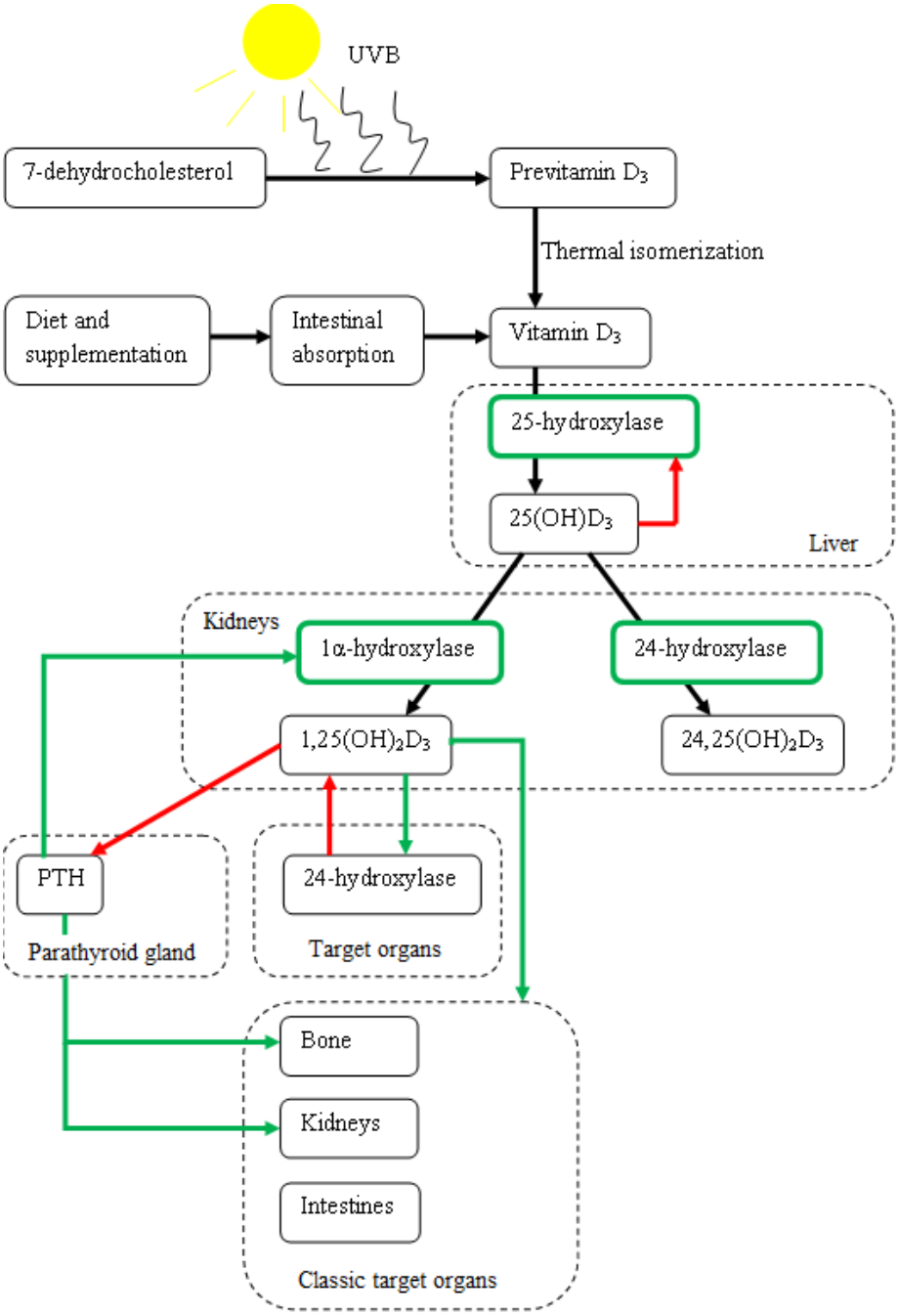

Figure 1: Regulation of classic vitamin D metabolism. Green arrows indicate a stimulation or production whereas red arrows indicate an inhibitory effect or inactivation. Illustration adopted from Janssen et. al. 2002 (124) with modifications based on literature and illustrations from Holick, 1994 (125), Holick, 2005 (32) and Galea et al., 2011 (25). PTH: Parathyroidhormone.

A specific receptor for $1,25(OH)_2D_3$, the vitamin D receptor (VDR), has been identified in almost all cells in the body and is essential to mediate the numerous actions of $1,25(OH)_2D_3$. The VDR is a ligand-dependent receptor belonging to the steroid-thyroid hormone nuclear receptor superfamily (25,38,42,43). The best known target tissues are bone, intestines and kidneys, but one of the more recently discovered targets is skeletal muscle tissue (38). When the metabolites of vitamin D reach the target cell they enter the cell and binds to the VDR

located in the nucleus (27). This complex forms a heterodimer with the Retinoid X receptor and binds to a vitamin D responsive element (VDRE) on a responsive gene, e.g. the genes for calcium binding protein or 24-hydroxylase (27). Subsequently, transcription and translation occurs whereby proteins from the specific gene are formed, e.g. calcium binding protein (27). Additionally, VDRs have been identified in the target cell membrane, which will be further elaborated in section *1.4 The effect of vitamin D in muscle tissue*.

The above described processes may be reduced with age. The cutaneous production of vitamin D_3 is impaired due to decreased levels of 7-dehydrocholesterol and reduced dermal clearance (18). Conflicting results exist on whether the intestinal absorption of vitamin D is decreased with age (44-46). *Harris et al., 1999* showed that 25(OH)D_2 serum levels increased less in older men compared to the increase in younger men following three weeks of daily 45 µg (1800 IU) vitamin D_2 supplementation (45), indicating a decreased intestinal absorption of vitamin D_2 and/or hepatic conversion into 25(OH)D_2 in the older men. Generally though, the hepatic conversion into 25(OH)D is thought not to be affected with age (18). However, severe liver disease or decreased liver function may result in reduced synthesis of 25(OH)D. Accordingly, it is unclear if aging has an effect on the synthesis and/or metabolism of 1,25$(OH)_2$D, which might depend on several factors, e.g. impaired renal function (18). Animal and human studies have shown that the responsiveness to 1,25(OH)D in certain tissues, like the duodenal mucosa, may be decreased with age (47,48). Additionally, it has been shown that elderly have decreased expression of VDRs in some tissues, including muscle tissue, which negatively influences on the action of vitamin D (49).

1.2 Vitamin D deficiency

For vitamin D to exert its optimal effect in the body, an optimal 25(OH)D serum level is required. In the summer half, exposure to sunlight for 5-30 minutes a few times a week is estimated to be sufficient, however, longer time of exposure induces no further contribution (28), as previously described. Insufficient exposure to sunlight and an increased metabolism of vitamin D due to e.g. low level of calcium intake or decreased absorption are both factors which can lead to vitamin D deficiency. This can further result in rickets and osteomalacia, which both increase the risk of fractures (38). Measurement of total 25(OH)D serum levels have emerged to be the best approach to assess an individual's vitamin D status (38,41,50), since it reflect both the endogen production of vitamin D_3 by ultraviolet radiation and the dietary intake of vitamin D_2 and D_3 (41,51).

The official recommendation by the Danish Health and Medicines Authority for individuals above 65 years is a daily dose of 10 µg (400 International Units (IU)) of vitamin D_3 (2). The Scientific Committee on Food in the European

Commission has established the upper recommended daily intake to be 50 µg (2000 IU) for adults (28), which is also the recommendation set by the Endocrine Society (52). However, The Institute of Medicine (IOM) has established a dose of 100 µg/d (4000 IU/d) to be safe (53). Literature accordingly shows that a serum level of 50-60 nmol/L of 25(OH)D_3 is associated with the lowest all cause-mortality risk, and that both serum levels under 10 nmol/L and above 140 nmol/L are associated with higher all cause-mortality risk (41,54). In a report on the dietary recommendations from 2011, The Institute of Medicine recommends a serum level of 50 nmol/L 25(OH)D to be sufficient for 97,5 % of the population in regards to bone health, which is the level most often considered sufficient, while a level of 125 nmol/L is considered not to cause toxicity (53). There is lacking consensus among experts on the optimal 25(OH)D serum level. One review found that a serum concentration between 90-100 nmol/L is the optimal for lower extremity strength in elderly (55). This suggests that the current recommended doses are insufficient for optimal muscle health and that recommended daily doses and serum levels in elderly may need to be reevaluated.

1.3 The aging muscle

With advancing age a decline in the amount of muscle mass and strength is observed together with a decline in the quality of the muscle fibers which is known as sarcopenia (56). The alterations in muscle function are seen from approximately 40 years of age (57). The morphological changes in the muscle fibers in association with aging are an overall decrease in amount of muscle fibers and muscle fiber size together with infiltration of fat and fibrous tissues into the skeletal muscle. The atrophy of skeletal muscle is both due to individual fiber atrophy together with a decline in total fiber count, predominantly affecting type II muscle fibers, also known as fast twitch fibers (58). These fibers are thought to be the fibers that are recruited first during rapid movements, e.g. to prevent a fall (59-61). The atrophy or loss of type II fibers are thought to be caused by loss of motor neurons innervating these fibers. De-innervated fibers may be re-innervated by slow motor units, and thereby transform into slow twitch fibers, which will increase the size of these motor units (61,62). This may all result in a reduction in the fine motor control, less controlled movements, muscle weakness and reduced postural control, and thereby result in an increased risk of falling. There are many proposed mechanisms behind the molecular basis of sarcopenia, besides the simplistic explanation of increased inactivity with age due to decreased motor demands. It is beyond the scope of this report to review them all. This report will primarily focus on how inflammatory cytokines are able to contribute to the aging process in muscles.

An increase in inflammatory cytokines has been observed with age. Studies in humans show that the pro-inflammatory cytokines elevated are predominantly tumor necrosis factor-α (TNF-α)

and interleukin (IL-) 6 (63-65). DNA mutations associated with age are a result of lifelong oxidative damage from immune processes, in addition to damage from oxidative metabolism, and may result in dysfunctional DNA, proteins and lipids, causing cell dysfunction (66,67). Since type II fibers contain a low level of mitochondria, they are thought to be less specialized in counteracting the oxidative damage (56). Dysfunctional cells release cytokines that will attract macrophages leading to further oxidative damage. Furthermore, the dysfunctional cells are believed to make the fibers more susceptible to injury (66,68). Accordingly, the extensive oxidative damage may as well be responsible for dysfunctional contractile proteins, including myosin heavy chains, and mitochondrial proteins, also observed with advancing age (69,70). Furthermore, the sarcoplasmic reticulum of aged rodents have been found to exhibit decreased ability to release calcium and also to be reduced in size (71).

During muscle regeneration, damaged skeletal muscle fibers undergo phagocytosis, autolysis and replacement of lost muscle fibers to repair the damage. Cytokines released from the inflammatory process stimulate satellite cells to fuse with damaged fibers and repair them, and thus, proliferate and differentiate into new repaired muscle fibers. However, if the cytokine response is increased, as described above, this may affect differentiation of satellite cells negatively and cause apoptosis of these. In this way increased levels of pro-inflammatory cytokines may have catabolic effects and contribute to muscle wasting and a decrease in muscle strength (72,73). A human study showed that satellite cells surrounding type II fibers primarily were affected (74) which also could contribute to the primary loss of type II fibers with age.

1.4 The effect of vitamin D on muscle tissue

The first link between vitamin D deficiency and muscles derives from very early observations that bone disease, e.g. osteomalacia and rickets, presented together with muscular weakness (75). Vitamin D deficiency has, besides muscle weakness, later been linked to impaired balance, increased risk of falling and muscle pain (6,76-78). In addition, vitamin D deficiency has been shown to mainly affect the weight-bearing muscles of the lower extremities, which plays an important role in balance (77). A clinical study in which muscle tissue biopsies were taken from osteomalacic individuals revealed main atrophy of type II muscle fibers (79). Accordingly, another study showed that the amount of type II fibers can be enhanced by vitamin D_2 supplements of 1000 IU per day (80). It is well established that $1,25(OH)_2D_3$ is able to exert indirect effects on muscle through systemic regulation of calcium and phosphate homeostasis, leading to moderations in intracellular sarcoplasmic concentration of these substances (81). However, there is also evidence that $1,25(OH)2D_3$ has direct effect on muscle, and it is only over the recent decades that the mechanism behind

this putative effect is starting to be unraveled. Studies on cultured myotubes and myoblasts have started to elucidate potential mechanisms on how 1,25(OH)2D_3 affects muscle cells, which will be described in the following.

1,25$(OH)_2D_3$ exerts its direct effect on skeletal muscle by binding to a VDR associated with the muscle cell. Two different kinds of muscle VDRs have been described; a nuclear receptor that mediates genomic actions and a membrane associated receptor that mediates non-genomic actions (82,83). There is, however, a lot of controversy in the literature about which effects are mediated by which pathway, and also the effects of the pathways may overlap (81,84-87). However, the pathways are often divided as outlined in the following, see *figure 2*.

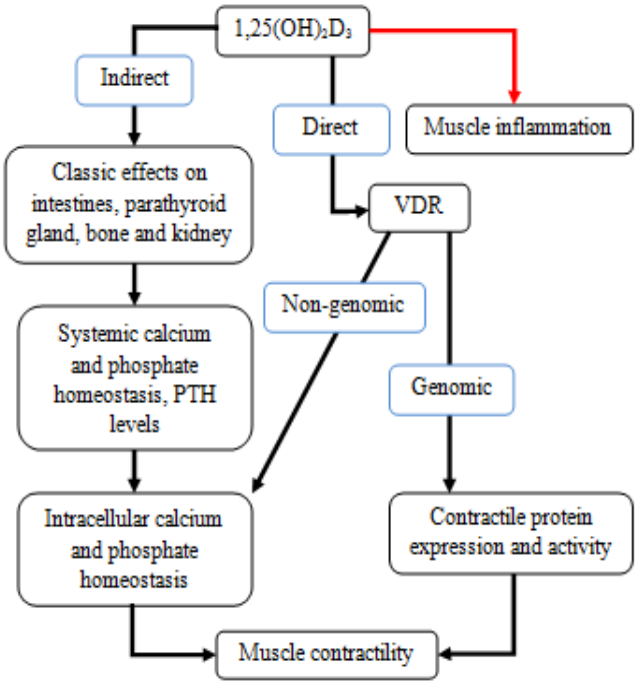

Figure 2: The effect of 1,25$(OH)_2D_3$ on muscle tissue. Red arrows indicate inhibition. The illustration with modifications is adopted from Girgis et al., 2013 (81). VDR: Vitamin D receptor, PTH: Parathyroid hormone.

1.4.1 Non-genomic actions

1,25$(OH)_2D_3$ is able to affect muscle cells through rapid non-genomic pathways initiated by a VDR located at the cell surface. As 1,25$(OH)_2D_3$ binds to the receptor the cellular effects can be observed within seconds to minutes (84). Still, it is not fully determined whether the membrane receptor is a separate receptor or if it is a translocation of the nuclear receptor (81,86). Non-genomic rapid effects are thought predominantly to include regulation of intracellular calcium and phosphate homeostasis in the muscle cell (81). Studies have found that as 1,25$(OH)_2D_3$ binds to the non-nuclear VDR it initiates a cascade of different pathways within the cell. This results in modulation of the intracellular calcium homeostasis by mobilization of calcium from the sarcoplasmic reticulum (SR) and via entry of calcium from extracellular space through voltage-depended calcium channels (VDCCs) and 'store-operated' calcium entry (SOCE) (81). These precise pathways are still controversial in the literature and need further elucidation. Additionally, 1,25$(OH)_2D_3$ and also 25$(OH)D_3$ have been shown to increase phosphate uptake and adenosine triphosphate (ATP) synthesis (88,89), thereby making more ATP available for the contraction process. These results are provided from *in vitro* and animal studies, however, a recent human study showed improved muscle energy metabolism with vitamin D_3 supplementation of vitamin D deficient young adults (90).

1.4.2 Genomic actions

Genomic effects are achieved less rapidly than non-genomic effects due to requirements of protein synthesis. For $1,25(OH)_2D_3$ to exert its effects it is transported to the nucleus by an intracellular binding protein, and subsequently $1,25(OH)_2D_3$ is able to bind to the nuclear VDR which regulates mRNA transcription and thereby protein synthesis in the muscle cell (84). Putative effects of activation of the nuclear receptor are mainly effects on expression of contractile proteins in the muscle cell, and differentiation and proliferation of myoblasts which will not be further elaborated here (84). Increases in muscle contraction have been observed in chicks without vitamin D deficiency compared to chicks that were deficient (91). Accordingly, two other studies reported decreases in components of the actin-myosin-troponin complex for vitamin D deficient rats and rabbits. However, a substance known to inhibit vitamin D was not able to render this effect (92,93). Additional research, including human studies, is needed on this matter.

1.5 The effect of vitamin D on cytokines

As described, literature suggests that low-grade inflammation and vitamin D deficiency is associated with sarcopenia (94), and it has been hypothesized that a higher level of inflammatory markers play a role in the functional decline of older individuals (73). In addition, *in vitro* and *in vivo* studies have shown that vitamin D_3 suppresses pro-inflammatory cytokines and increases anti-inflammatory cytokines (95,96). One *in vitro* study has shown that $1,25(OH)_2D_3$ is able to up-regulate the synthesis of IL-10 and induce IL-10 receptor expression (95,97). Clinical studies, investigating the effect of vitamin D on anti-inflammatory cytokines (95,98) have shown supportive results to this *in vitro* study. *Schleithoff et al. 2006* showed an increase in IL-10 in patients suffering from congestive heart failure when given supplements of vitamin D_3. Furthermore, their results indicated that vitamin D_3 supplementation is able to prevent an increase in the serum concentration of TNF-α (95). A former study conducted by *Müller et al. 1992*, showed evidence that $1,25(OH)_2D_3$ dose-dependently inhibits the production of IL-1α, IL-6 and TNF-α by human blood monocytes (96). Supporting that vitamin D stimulates IL-10, *Zittermann et al. 2004* obtained data from infants indicating a positive correlation between this anti-inflammatory cytokine and 25(OH)D levels (99). To support all of these findings, it is necessary to do more research on vitamin D and inflammatory cytokines in humans, including elderly people, an area where few studies have been performed.

1.6 Summary of vitamin D and aging

In summary, aging is believed to affect the cutaneous production, intestinal absorption, metabolism and action of vitamin D. In addition, age-related changes in muscles are observed. In elderly inflammation may cause sarcopenia and decreased muscle strength through the mecha-

nism of oxidative damage to DNA. The damage may result in dysfunctional cells and proteins including both contractile- and mitochondrial proteins and also proteins associated with calcium release from the SR. This may all contribute to decreased ability of the muscle to contract and also lead to apoptosis of dysfunctional cells. Furthermore, the increased inflammatory response may be able to affect differentiation of satellite cells negatively, causing impaired muscle repair following contraction-induced muscle injury further contributing to muscle weakness. This response is thought to be most pronounced in type II fibers since they are less specialized in coping with oxidative stress. Since pain is one of the cardinal features of inflammation, this chronic low-grade inflammation could also lead to muscle pain and/or decreased pain thresholds in muscles.

As mentioned, $1,25(OH)_2D$ can affect muscles in both a direct and an indirect way. Systemic calcium and phosphate homeostasis is disrupted through impaired conversion of vitamin D and decreased expression of VDR with age. This will affect the intracellular levels of calcium and phosphate resulting in decreased contractility. Furthermore, studies have proposed that $1,25(OH)_2D_3$ acts directly on muscle VDRs, and thereby increases the calcium concentration within the muscle cell via enhanced calcium release from the SR and calcium entry from extracellular space through SOCE and VDDCs. It has further been suggested that the increased amount of intracellular calcium together with ATP is able to affect muscle function positively by making more of these substances available for muscle contraction. Furthermore, $1,25(OH)_2D_3$ is believed to be able to increase the amount of contractile proteins, e.g. actin and myosin filaments, in the muscle. These effects may be mediated through genomic or non-genomic pathways. Additionally, it is believed that vitamin D_3 has an inhibitory effect on pro-inflammatory cytokines, while it has a stimulating effect on anti-inflammatory cytokines. Together, this supports that vitamin D_3 may suppress the low-grade inflammation observed in elderly people, and thereby lower the degree of oxidative stress responsible for dysfunctional cells and proteins. Also, vitamin D_3 may lower cytokines responsible for impaired muscle repair following injury through inhibition of satellite cells. In this way it is suggested that vitamin D_3 is able to counteract some of the negative impacts on muscle form, function and metabolism caused by aging, especially if vitamin D deficiency is a significant part of aging.

1.7 Vitamin D and muscle function

As previously described, falls in elderly are associated with muscle atrophy and decreased muscle strength (5,100), and consequently is thought to increase risk of falling. Studies have found low 25(OH)D serum levels to be associated with decreased muscle strength, including muscle strength in lower extremities (101-103). Various interventional studies have examined the effect of vitamin D on muscle function (for

review see *Muir and Montero-Odasso, 2011*, *Rejnmark, 2011*, *Latham et al., 2003* (126)). There are controversial findings in the literature, as the majorities of the studies regarding the effects of vitamin D on muscle strength are unable to conclude that vitamin D supplementation is able to increase muscle strength in elderly, however, there are also studies showing positive results for vitamin D (104). A meta-analysis found a beneficial effect for higher doses (800-1000 IU) of vitamin D compared to lower doses (< 20 µg (800 IU)) on muscle strength and balance but not on gait (11). A review by *Gillespie et al. 2012*, suggests that individuals with low 25(OH)D baseline levels will benefit more from treatment with vitamin D (105). In relation to muscle strength, a meta-analysis proposed that elderly with a 25(OH)D serum level below 20 nmol/L have the most benefit from vitamin D supplements (106). As a large percentage of Danish adults above the age of 65 suffers from vitamin D insufficiency (7,8), Danish elderly would consequently be an appropriate study population for an interventional study.

2 Aim of Study

The aim of this study was to compare the effect of a high daily dose of 50 µg (2000 IU) vitamin D_3 compared with placebo on muscle strength and functional mobility after an intervention period of 12 weeks in Danish volunteers above the age of 65.

2.1 Hypothesis

It was hypothesized that vitamin D_3 is able to increase muscle strength and functional mobility by; making additional calcium and ATP available for muscle contraction, up-regulating synthesis of contractile proteins and reducing inflammation in the muscle.

3 Methods

Originally, the project group wanted to investigate the combined effect of vitamin D supplementation and a training regime of one hour two times a week. This training regime would focus on strengthening of the lower extremities and improvement of balance, see *appendix A*. This was, however, unfeasible due to limited time, resources and lack of volunteers. Instead the effect of a high dose of vitamin D was investigated in the following study design.

3.1 Study design

The study was conducted as a randomized parallel double-blinded placebo-controlled study, illustrated in *figure 3*. Subjects were randomized to receive either a daily dose of 50 µg (2000 IU) vitamin D_3 or placebo.

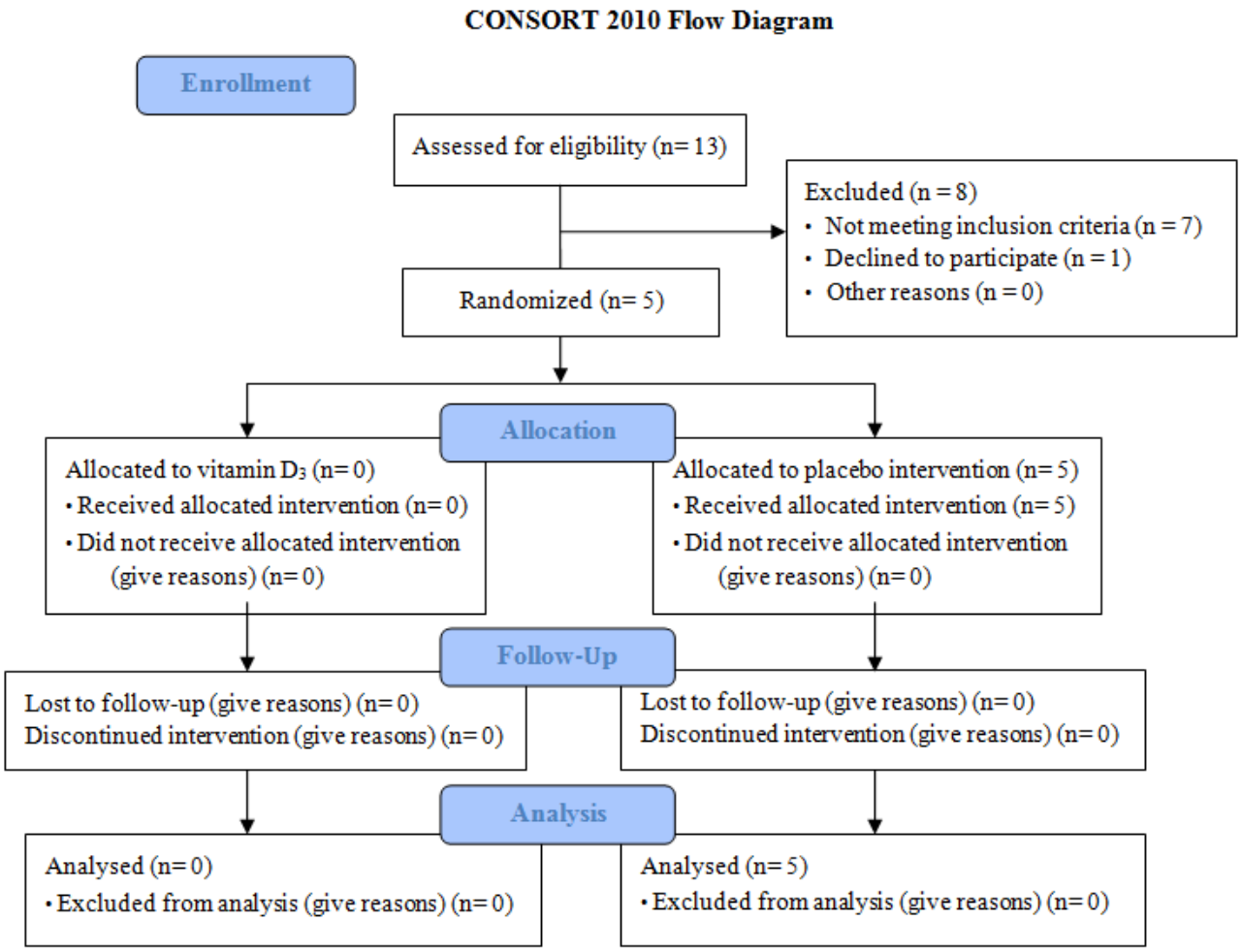

Figure 3: CONSORT Flow Diagram illustrating the parallel study design with the phases; enrollment, allocation, follow-up and analysis.

At a preliminary visit and a screening visit it was decided, based on the inclusion and exclusion criteria, whether the subject could or could not participate in the study. The treatment period was 12 weeks, and consisted of two visits approximately 84 days apart (baseline and post-treatment), +/- 14 days, see *figure 4*. At both visits the subjects were evaluated on mobility, muscle strength, balance, pressure pain threshold (PPT), and quality of life measures, including a questionnaire regarding the subjects fear of falling. Furthermore, a blood sample analyzing 25(OH)D serum levels was obtained during both visits. The subjects received a phone call from the investigators midway through the treatment period (approximately at day 42) to check up on study medication compliance, to ensure the subjects were remembering to fill out fall diaries in case of falls and to check for adverse events.

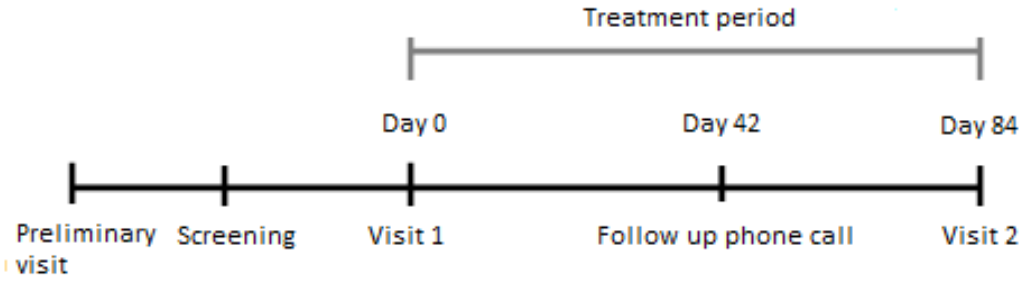

Figure 4: Overview of the study period, including the screening session, visits, follow-up call and treatment period. At visit 1 and 2 baseline measures and post-treatment measures were obtained, respectively.

3.2 Subjects

5 volunteers, three females and two males (mean body mass index (BMI) 26.8 ± 3.1), above the age of 65 (mean age 72.8 ± 5.5 years) were recruited for this pilot study through advertisement at senior societies and fitness clubs, physiotherapists and Aalborg University Hospital's Geriatric Ward. The recruitment process took place between January and March 2014. Inclusion criteria were; subjects above the age of 65 years, a history of at least one fall in the previous 12 months and/or that the subjects reported his/her balance as average or poor on a scale with the levels good, average or poor. Exclusion criteria were; a daily intake of vitamin D above 35 µg (1400 IU), inability to stand without support, drug addiction defined as the use of cannabis, opioids or other narcotics, or lack of ability to cooperate. All participants gave their written informed consent prior to participation in the study and the study was approved by the Ethics Committee of the North Jutland Region, Denmark (N-20130069). The study was conducted at Center for Clinical and Basic Research in Aalborg, Denmark.

3.3 Treatment

The subjects were randomized to receive either a daily dose of 50 µg (2000 IU) vitamin D_3 (as two tablets of 25 µg) or matching placebo (D3 Pharmacy ApS, Bispensgade 22, 9000 Aalborg, Denmark) for 12 weeks. Both the study participants and the investigators of the study were blinded to the treatment allocation. At visit 1 the study medication was handed out to the patient and remaining tablets were returned at visit 2, where compliance was evaluated through remaining pill count. The participants were asked about adverse events at both the midway phone call and at visit 2. The placebo tablets were visually identical to the vitamin D_3 tablets, but contained no therapeutic effect. All study medication was packed in uniformly labeled pill bottles holding the subject's randomization number, so that vitamin D_3 therapy was indistinguishable from placebo. Labeling of the study medication and randomization was carried out by a person not responsible for dispensing the study medication to study participants.

3.4 Outcome measures

All measurements were performed at visit 1 (baseline) and visit 2 (post-treatment). Information about general health status, including height, weight, smoking habits and physical activity level, were noted at visit 1 in order to be able to adjust for confounders in the statistical analyzes. Height and weight were used to calculate BMI. In addition, current medication, dietary supplementations and illnesses were noted at visit 1. Blood samples were also drawn at both visits. During the treatment period participants were asked to fill out fall diaries at home in case they experienced a fall.

3.4.1 Primary outcomes

3.4.1.1 Timed Up and Go Test

Mobility functioning was tested by the Timed Up and Go (TUG) Test. The test is developed for the elderly population and measures the time it takes for a person to get up from a chair with armrests (seat height approx. 43-47 cm), walk 3 meters, turn, walk back to the chair and sit down again (107).

To assess the reliability of this test a pilot study was performed on healthy adults between the ages of 24-53 years revealing a coefficient of variation (CV) of 3.66 %. The results from the pilot study are shown in *table 1* below.

Subject	Trial 1 (sec.)	Trial 2 (sec.)
1	6.4	6.2
2	5.1	4.6
3	4.4	4.4
4	5.6	5.1
5	5.7	5.7
6	8.4	8.1
7	4.9	5.1
8	5.2	5.3
Mean		5.64
SD		0.21
CV (%)		**0.037 (3.66)**

Table 1: Results from the pilot study performed in order to calculate the coefficient of variation (CV) for the Timed Up and Go (TUG) test and to estimate the sample size. SD: Standard deviation.

3.4.1.2 Muscle strength

Maximal isometric contraction strength (maximal voluntary contraction (MVC)) for the knee extensors (quadriceps femoris muscle) was assessed by a hand-held myometer (Myometer D60107MK1 model, Penny & Giles Instrumentation Ltd., Christchurch, Dorset, UK) at baseline and post-treatment for the subject's dominant or preferable leg. Same leg was used at both visits. The myometer was placed approximately 8 cm proximally to the ankle joint on the tibia, and the subjects were instructed to exert their maximal force against the spreader applicator for 5 sec. and then stop. The subjects were given one test round, and subsequently the mean of three measurements was used for analysis. The subjects were verbally encouraged by the investigator to exert maximum effort for five seconds followed by a pause of 10 seconds.

In order to validate the hand-held myometer a standardized 100 kPa weight was placed on top of the vertical-held myometer. The mean of ten measurements were 1.2 Kg-F; meaning the myometer has a systematic error of 0.2 Kg-F. This was repeated with a heavier weight revealing the same systematic error.

To assess the reliability of this test of muscle strength, a pilot study was performed on healthy adults between the ages of 24-55 years, revealing a CV of 7.08 %. The results are shown in *table 2* below.

Subject	Trial 1 (Kg-F)	Trial 2 (Kg-F)
1	35.5	37.5
2	28.1	31.7
3	29.5	26.5
4	34.3	35.1
5	29.7	25.7
6	9.0	6.8
7	13.5	13.6
8	20.0	18.7
Mean		24.70
SD		1.75
CV (%)		**0.071 (7.08)**

Table 2: Results from a pilot study performed in order to calculate the coefficient of variation (CV) for the maximal muscle strength (MVC) test. SD: Standard deviation.

3.4.2 Secondary outcomes

3.4.2.1 Berg's balance test

Berg's Balance Scale quantifies functional balance and consists of 14 exercises, each rated by the investigator on a scale from 0 (impossible) to 4 (completely independent execution). The 14 different exercises include; sitting to standing, standing unsupported, standing to sitting, sitting without support, transferring from chair to bed, standing with eyes closed, standing with feet together, reaching forward with straight arms, picking an object up from the floor, twisting and looking back, turning 360 degrees, putting feet alternately on stairs, tandem stand and one leg stand, see *appendix C*.

3.4.2.2 Pressure Pain Threshold

PPTs for the m. Gluteus Medius were assessed by means of pressure algometry (Somedic algometer, Somedic AB, Hörby, Sweden). The pressure applied was with a velocity of 30kPa/s with a 1 cm diameter rubber probe. The subject was instructed to press a hand-held trigger button when the subject perceived the pressure as painful. The mean maximum pressure of three measurements recorded at each point was used for later analysis. The three measurements were not to deviate more than 25 % from their mean. In case of deviation a new single measurement was taken. Two test points on the gluteus medius muscle were identified for the dominant or preferred side. The same side was assessed at both visits. Test point one (TP1) were located 2.5 cm lateral to the posterior superior iliac spine and 2.5 cm distal to lip of the iliac crest. The second test point (TP2) was located 2.5-5 cm lateral to TP1 just under the apex of the iliac crest. At visit 1 the distance between TP2 and TP1 was noted to ensure relocation of TP2 at visit 2.

To assess the reliability of this test as well, a pilot study was also performed on healthy adults between the ages of 18-52 years revealing a CV of TP1 of 28.3 % and a CV of TP2 of 13.4 %. The results are shown in *table 3* below.

	Trial 1		Trial 2	
Subjects	**TP1**	**TP2**	**TP1**	**TP2**
1	166.33	153.33	194.67	204.0
2	233.33	228.00	256.00	197.0
3	86.33	78.33	110.00	105.33
4	479.67	447.00	392.67	505.33
5	197.33	137.00	161.67	114.33
6	129.00	236.00	126.67	154.33
7	205.67	243.67	186.00	262.33
8	476.7	241.3	309.7	306.3
Mean TP1				227.7
Mean TP2				230.1
SD TP1				64.5
SD TP2				30.8
CV TP1 (%)				**0.283 (28.3)**
CV TP2 (%)				**0.134 (13.4)**

Table 3: Results from a pilot study performed in order to calculate the coefficient of variation (CV) for the pressure pain threshold (PPT). TP1/2: Test point 1/2, SD: Standard deviation.

3.4.2.3 Quality of life measurements

The subjects' health related quality of life and function was assessed by EQ-5D-5L (Danish version 2.0), see *appendix D*. The questionnaire is short and consists of five multiple choice questions followed by an EQ visual analog scale (VAS) to evaluate the subject's perception of his/her general health and quality of life. Fear of falling was evaluated though the Falls Efficacy Scale-International (FES-I) questionnaire (Danish version), see *appendix E*. This questionnaire consists of 16 questions regarding the subject's fear of falling when performing or imagining to performing normal daily and domestic activities.

3.4.2.4. Fall diary

In case a subject would experience a fall in the study period they were asked to fill out a fall diary regarding the conditions of the fall; including time of day, in what context the fall occurred, the influence of medications and the consequences of the fall, see *appendix F*.

3.5 Biochemistry analysis

Venous blood samples were obtained at baseline and post-treatment and analyzed for total 25(OH)D ($25(OH)D_2$ and $25(OH)D_3$) serum levels by using an electrochemiluminescence-binding assay. The Elecsys Vitamin D Total analysis utilizes a VDBP as a capture-protein to bind the $25(OH)D_2$ and $25(OH)D_3$. Sampling of the blood was performed by educated staff at CCBR Clinical Research A/S in Aalborg. Samples were cooled down to 2-8 ^{o}C, protected from light and transferred to the Clinical Biochemical Department at the University Hospital of Aalborg for analysis. The analysis was performed two times on the same sample to minimize uncertainties of measurements. Tubes used for sampling were 2 mL (minimum 0.5 mL) hemolytic-free Li-heparin plasma tubes, tube type 8. The limit of detection was defined as 3.00-70.0 ng/mL (approximately 7.5-175.0 nmol/L) and values below this limit were stated

as < 3.00 ng/mL (approximately < 7.5 nmol/L). A blood sample from each subject was obtained at baseline and post-treatment and precision was expressed as CV 1.7-7.8 % in intra-assay and CV 2.2-10.7 % in inter-assay, see *appendix G*.

3.6 Statistics

Residuals of baseline measures and post-treatment measures for outcome measures were checked for normality by plotting the data into histograms and Q-Q plots. Repeated measure analysis of covariance (ANCOVA) was intended to be used to compare the two groups with the between-subjects factor 'time' (1: baseline, 2: post-treatment). Treatment was to be included in the analysis as a between subjects-factor, and age, gender and BMI were to be added as covariates. The potential baseline differences between the treatment groups at baseline were to be examined using a two-tailed unpaired t-test. All statistical analyses were made with the statistical software SPSS (SPSS inc. IMB Company©, v. 20) using a significance level of 5 %.

3.6.1 Power calculation

Estimation of the sample size was based on the TUG test measurements from the pilot study on healthy volunteers performed by the investigators, data are presented in *table 1*, see *3.4.1.1 Timed Up and Go Test*. To estimate the sample size following equation was used:

$$N = \left(C_{2\alpha} + C_{\beta}\right)^2 \times \frac{(2 \times SD)^2}{\Delta^2}$$

The $C_{s\alpha}$ is the 2α quantile in the normal distribution and C_{β} is the β quantile in the normal distribution. SD is the standard deviation and Δ is the desired smallest relevant clinical difference detected.

With Δ set to 0.2 seconds corresponding to 3.5 % for TUG, α set to 0.05 (corresponding to a significance level of <0.05) and β set to 0.20 (corresponding to a power of 80 %), this will give a table value of 7.84. The values inserted in the formula provide the following:

$$N = 7.84 \times \frac{(2 \times 0.27)^2}{(0,2)^2}$$

$$N = 57.15$$

This calculation suggests that 57 subjects were sufficient to detect this difference between the groups. Including an expected drop-out rate of 15 % it was estimated that 68 patients were needed to be recruited.

Due to limited resources it was not possible to recruit the necessary number of subjects to obtain the sufficient power for this study. Instead a pilot study was performed on five volunteer subjects who met the inclusion and exclusion criteria.

4 Results

All five subjects completed the study, and all were allocated placebo treatment. Consequently, it was not possible to compare means of the two treatment groups. Instead paired t-tests were performed for each outcome measure between baseline and post-treatment.

4.1 Primary outcomes

Measurements from the TUG test improved from 8.2 seconds to 7.3 seconds (-0.9 sec; -

11%) following the treatment period. This improvement was found to be significant (p = 0.05). The MVC measurements showed a significant (p = 0.01) improvement from 14.6 Kg-F at baseline to 19.2 Kg-F post-treatment (+4.6 Kg-F; +31.5%). The means and p values for the primary outcomes are presented in *table 4* below.

Characteristics	Baseline	Post	*p* Value
TUG (sec.)	8.2 ± 2.4	7.3 ± 2.6	0.05*
MVC (Kg-F)	14.6 ± 3.9	19.2 ± 4.2	0.01*
PPT TP1 (kPa)	409.8 ± 208.6	295.0 ± 136.7	0.06
PPT TP2 (kPa)	326.5 ± 175.2	284.0 ± 79.6	0.50
Berg's Balance Scale (score)	48.0 ± 8.6	51.0 ± 9.6	0.11
FES-I (score)	22.6 ± 5.4	21.2 ± 2.8	0.44
EQ-5D-5L (score)	0.789 ± 0.128	0.829 ± 0.121	0.07
EQ VAS (score)	73.0 ± 24.4	84.0 ± 13.9	0.42
25(OH)D (nmol/L)	79.6 ± 57.7	89.4 ± 63.5	0.02*

Table 4: Characteristics of subjects at baseline and post-treatment (mean ± SD).TUG: Timed Up and Go test, MVC: Maximal Voluntary Contraction, PPT: Pressure pain threshold, TP1/2: Test point 1/2, FES-I: Falls Efficacy Scale-International, SD: Standard deviation.

4.2 Secondary outcomes

The threshold for TP1 decreased from 409.8 kPa at baseline to 295.0 kPa post-treatment (-114.8 kPa; -28%), while the threshold for TP2 decreased from 326.5 kPa at baseline to 284.0 kPa post-treatment (-42.5 kPa; -13%). Thus, the threshold for both test points decreased following treatment, however, the differences between the sessions were not statistically significant. The Berg's Balance Scale score improved non-significantly from 48.0 at baseline to 51.0 post-treatment (+3; +13%). In addition, no significant differences were found in either the FES-I scores or the EQ-5D-5L questionnaires including the EQ VAS score. In the FES-I a slight decrease in the concerns of falling was observed from baseline 22.6 to 21.2 post-treatment (-1.4; -6.2%). The EQ-5D-5L and EQ VAS scores increased from 0.789 to 0.829 (+0.04; +5.1%) and from 73.0 to 84.0 (+11; +15.1%), respectively. The 25(OH)D serum level increased significantly from 79.6 nmol/L to 89.4 nmol/L (+9.8 nmol/L; +12.3%) following the treatment period (p = 0.02). All means and p values for the secondary outcomes are also presented in *table 4* above.

One subject reported two falls during the study period. No other subjects experienced any falls.

4.3 Correlations

The effect of 25(OH)D serum levels on the outcome measures were evaluated by Pearson's correlation coefficient. A significant correlation was found between 25(OH)D status and PPT for TP1 post-treatment (r = 0.913, p = 0.031). In addition, 25(OH)D status and PPT for TP2 post-treatment also correlated significantly (r = 0.964, p = 0.008). No other correlations with 25(OH)D serum levels and remaining outcomes were found. Furthermore, the effects of age, gender and BMI on the outcome measures were also evaluated by Pearson's correlation coefficient. A significant correlation between age and TUG measurements post-treatment was found (r = 0.932, p = 0.021), while no significant correlations were found between gender and any of

the outcome measures. A number of outcome measures correlated significantly with BMI at baseline; TUG test (r = 0.889, p = 0.044), Bergs Balance Scale (r = -0.924, p = 0.025), FES-I (r = 0.978, p = 0.004) and EQ VAS score (r = -0.992, p = 0.001). In addition, the measures from Berg's Balance Scale post-treatment also correlated significantly with BMI (r = -0.962, p = 0.007).

4.4 Adverse reactions

One subject reported muscle soreness which prevented the subject from visiting the gym as usual. Since the subject received placebo treatment this is assessed not to be attributed to the treatment. No other adverse events were reported.

5 Discussion

The primary outcomes, MVC and TUG measurements, showed significant improvements from baseline to post-treatment, which was unexpected since no subjects received any active treatment. These improvements may be due to placebo effect and due to subjects' eagerness to improve at the second visit. In the MVC test the subjects were, however, encouraged to exert their maximal effort at both visits and the subjects were accordingly given the same instructions at both visits. The improvement may also be explained by a learning effect since the subjects were more familiar with the tests at the second visit. This could have been avoided by letting the subjects attend a training session prior to the study. Additionally, it cannot be precluded that the subjects' daily activity level were increased during the treatment period due to more sunny weather in the spring inviting to more outdoor activity. Furthermore, increased awareness of their health status may also lead to increased physical activity. For secondary outcomes, no significant differences were observed between baseline and post-treatment except for the 25(OH)D serum levels, which were significantly increased from baseline to post-treatment. Since the subjects did not receive any active treatment this change could be caused by change in seasons. Additionally, two of the subjects had a daily intake of vitamin D supplementation between 25-35 µg at the inclusion and during the treatment period, which could contribute to the increased 25(OH)D serum levels if their 25(OH)D serum levels had not reached a steady state when attending this study. A significant finding in this study was that 25(OH)D status correlated positively with the PPT, which could be due to a anti-inflammatory effect of vitamin D_3. This finding was, however, only evident post-treatment. A non-significant tendency for lower threshold at the second visit was observed suggesting that the subjects performed more accurate at the second visit due to a learning effect. Some subjects had difficulties identifying when the pressure was perceived as painful, which supports the notion that the subject should attend a training session prior to the first visit. To our best knowledge this is the first study investigating

the association between 25(OH)D serum level and the PPT in elderly people. It should, however, be kept in mind that this study has low power due to a small sample size, but this trend should be evaluated further in larger scale studies.

If the subjects of this study were randomized to receive vitamin D_3 it was expected that their 25(OH)D serum levels would have been higher after the treatment period. It has been established that 25(OH)D serum levels increase approximately 0.7 nmol/L for each 1 µg vitamin D_3 given as an additional daily oral input (108). In this study a dose of 50 µg vitamin D_3 was intended to be given daily why it would be expected that the 25(OH)D serum levels in the subjects would increase approximately 35 nmol/L. Since this increase is dependent on dose, weight and prior 25(OH)D serum level, this assessment can only be considered an estimate. According to an equation composed by *Barger-Lux et al. 1998 (109)*, the project group estimated that 50 µg/d vitamin D_3 would increase 25(OH)D serum levels with 45.65 nmol/L after 8 weeks of treatment based on mean weight 77.16 kg of the subjects in the present study. As this increased 25(OH)D serum level were expected to continue rising in the four remaining weeks, unless a steady state was achieved, all subjects were, according to this equation, expected to have post-treatment 25(OH)D serum levels above the recommended levels and even higher.

Following vitamin D_3 supplementation it was expected that the subjects would improve in muscle strength and functional mobility, which in this study was assessed by TUG and MVC measurements. This improvement was hypothesized to be caused by vitamin D which is able to increase intracellular calcium, ATP synthesis and contractile proteins in muscle fibers and thereby impacting muscle contractility positively. Furthermore, by suppressing the pro-inflammatory cytokines, the potential inflammatory-induced pain in elderly was thought to decrease, which in time will be followed by less inactivity in the elderly. Subsequently, more physical activity further induces muscle strength and concurrently the inhibition of inflammation minimizes muscle weakness caused by the oxidative damage of the DNA. Thus, this may cause a decrease of falls in the elderly, since muscle weakness has been identified as a main risk factor for falling in elderly (110,111). Accordingly, time to complete the TUG test has been positively associated with falls, however, the predictive value of the test is controversial (112,113). One review suggests that the TUG test has more value for frailer elderly (113). Current studies have shown controversial results in regard to the effect on vitamin D on muscle strength and functional mobility in elderly. Some studies reported improved results after vitamin D supplementation (114,115), while others have shown no improvements (116-118). The controversy in the literature may therefore be due to heterogeneity in study designs includ-

ing dosing regimen in addition to low methodological quality (104). For example some studies investigated vitamin D_3 in combination with calcium supplementation, which may not give a precise result on vitamin D_3. As described in *1.7 Vitamin D and muscle function*, elderly suffering from vitamin D insufficiency may have more benefit from vitamin D supplementation. However, most studies do not include subjects based on their 25(OH)D serum level. Accordingly, a study by *Zhu et al., 2010* investigated the effect of 25 µg vitamin D_2 and found significantly improved muscle strength in the lower extremities in subjects with a mean baseline 25(OH)D serum level of 44 nmol/L and with low baseline muscle strength (119). It should, however, be noted that muscle strength did not increase for knee extensor, but for all other muscle groups in lower extremities, that were examined. The same study also reported a significant improvement in TUG measurements (119). Thus, the project group in this study would expect a similar increase in muscle strength following active treatment for insufficient subjects.

In future studies the project group would recommend the use of a fixated dynamometer instead of the hand-held myometer as utilized in this study, which would insure higher validity and reliability of the measurements. In this study, this disadvantage was minimized by fixating the investigator between the subject and a wall. Though, a CV of only 7.08 % was found on a small reproducibility study on healthy volunteers. It was learned that the edges of the spreader applicator were perceived as painful for some subjects during repeated measurements, which could result in a decreased MVC. However, since the subjects only were to exert their MVC for 5 seconds this is not believed to be of great significance. In addition to 25(OH)D serum level, the remaining secondary endpoints were accordingly expected to improve with vitamin D_3 supplementation. It has been demonstrated that lower extremity muscle strength positively is associated with balance and gait (120), The Berg's Balance Scale is a subjective measure of balance, and it would be more attractive to utilize an objective measure, e.g. a force plate. As vitamin D_3 is expected to decrease the low-grade inflammation in muscle tissue observed in elderly, it was expected that the PPT measurements would improve and thereby decrease. Studies have shown conflicting results between vitamin D status and pain (121), however, one study investigated the effect of vitamin D on PPT measurements and showed a positive result (122). As pain is a subjective matter it can be difficult to evaluate the threshold of this, however, the CV in the present study was 28.3 % and 13.4 % for TP1 and TP2, respectively.

Other general limitations were uncovered in this study. Only five subjects attended this study, which resulted in a low power. All subjects received placebo treatment as a result of randomization, however, no reason was found to suspect that the randomization was unsuccess-

ful. The subjects included in the study could be characterized as well-functioning, and mostly performed well in the physical tests. Therefore, it could be suggested that the inclusion criteria should be altered to target more frail elderly. In addition, two of the five subjects already received vitamin D supplementations (25-35 µg) before the study, and thus, between subjects variation of 25(OH)D serum levels was observed both at baseline and post-treatment. This could have been avoided by changing the exclusion criteria so that no supplementation or a maximum daily dose of 10 µg vitamin D was basis for exclusion. Consequently, it may be beneficial to analyze blood samples for 25(OH)D serum levels before including subjects in order to exclude subjects if their 25(OH)D status was considered adequate in accordance with present recommendations, i.e. a 25(OH)D serum level > 50 nmol/L. Supplementary, this study runs over 12 weeks, which is a limited period and it might not be sufficient to attain a steady state of 25(OH)D serum level. However, one study observed that both 25 µg/d and 100 µg/d vitamin D_3 reached a steady state of 25(OH)D serum levels after approximately 90 days of supplementation in insufficient healthy adults (123). Another important limitation is that the project group did not have the required resources to conduct the original study design, which was to investigate the effect of a 2 x 2 factorial design with a high dose vitamin D_3 supplementation and weekly strength training, allowing to investigate a potential synergic effect between vitamin D_3 and strength training.

One of the major strengths of this study was the study design which was a randomized double-blinded placebo-controlled design. This type of design eliminates a lot of bias, which would have been observed with a more simple design. Furthermore, the project group has investigated the reliability of the primary outcomes and PPT prior to the actual study conduct. Additionally, the validity of the myometer was partly evaluated.

If future research establishes that vitamin D supplementation has positive effects on muscle strength, function and/or pain, and thereby can decrease the risk of falling among elderly, this is a very simple, low-risk and inexpensive intervention with great clinical potential. Accordingly, if this intervention decreases the risk of falling, it will simultaneously decrease fall-related expenses, counting expenses related to treatment of fractures and to hospitalization, and the costs of home care or institutionalization that may be required after a fall. Hence, increased focus on vitamin D insufficiency in order to supplement insufficient individuals to decrease the risk of falling is a cost beneficial approach which should become a public health priority.

6 Conclusions and recommendations

Due to a low number of subjects and the course of randomization in this study it was not possible to neither confirm nor reject the hypothesis

that vitamin D_3 is able to improve muscle strength and functional mobility in elderly after an intervention period of 12 weeks. It is, however, recommended that future studies on this subject should be well-powered and utilize objective and well-validated outcome measures whenever possible. Furthermore, studies should include elderly subjects that are low-functioning and vitamin D insufficient, i.e. 25(OH)D serum levels < 50 nmol/L, at baseline. Additionally, the association between 25(OH)D and the PPT in elderly should be further evaluated in future large-scale studies.

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