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Title: The effect of high-dose vitamin D supplementation on muscular function and quality of life in postmenopausal women – a randomized controlled trial.

Short title: Vitamin D and muscle

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Abstract

Objective: Observational studies have suggested positive associations between serum 25-hydroxyvitamin D (25(OH)D) levels and muscular strength, balance and quality of life. Our aim was to examine whether high-dose vitamin D supplementation would improve these measures as compared to standard-dose vitamin D, as well as the possible muscular effects of Single Nucleotide Polymorphisms (SNPs) in genes encoding vitamin D-related enzymes.

Design: A 12-month randomized, double-blind, controlled trial where the participants received daily elemental calcium (1,000 mg) plus vitamin D_3 (800 IU). In addition, the participants were randomized to receive either capsules with vitamin D_3 (20,000 IU) or matching placebos to be taken twice a week.

Patients: 297 postmenopausal women with osteopenia or osteoporosis.

Measurements: Muscle strength (handgrip and knee extensor strength), balance (tandem test) and quality of life (EQ-5D) were measured at baseline and after 12 months. The subjects were genotyped for SNPs related to vitamin D metabolism.

Results: Of the 297 included women, 275 completed the study. Mean serum 25(OH)D levels dramatically increased in the high-dose group (from 64.7 to 164.1 nmol/l; $p<0.01$), while a more moderate increased was observed in the standard-dose group (from 64.1 to 81.8 nmol/l; $p<0.01$). There was no significant difference between the groups in change in muscular strength, balance or quality of life over the intervention period. Polymorphisms in rs3829251 (located in the 7-dehydrocholesterol reductase gene) was associated with muscle strength and treatment effects.

Conclusion: One-year treatment with high-dose vitamin D had no effect on muscular strength, balance or quality of life in postmenopausal women with osteopenia or osteoporosis as compared to standard-dose. The association between rs3829251 and muscle strength needs confirmation in other populations.

Introduction

The effects of vitamin D on calcium homeostasis and its importance for skeletal health are well-known [1]. In recent times, there has been an increased focus on non-skeletal effects of vitamin D, following the discovery of vitamin D receptors (VDRs) and the vitamin D activating enzyme 1-alfa-hydroxylase in extraskeletal tissues [2]. Observational studies have reported associations between higher serum 25-hydroxyvitamin D (25(OH)D) levels and lower risk of multiple pathological conditions[2]. It is of note, however, the general lack of data from randomized controlled trials (RCTs) confirming the positive effects hypothesized from the descriptive studies [2; 3] .

There has been conflicting data regarding the presence of VDR in muscle cells, which may be explained by different laboratory approaches. VDRs were detected in mice muscle cells by using highly specific antibodies in combination with a hyperosmolar lysis buffer able to release VDR bound to DNA [4]. In addition, the expression of VDR was higher in younger than in adult mice, and more pronounced in muscle cells precursors than in mature muscle cells, expression trends that were previously reported for human muscle cells [4; 5]

In observational studies, muscle strength and balance have been associated with serum 25(OH)D levels [6; 7]. A recent systematic review and meta-analysis reported a weak positive benefit of vitamin D intervention on global muscle strength, with a stronger benefit in adults with baseline serum 25(OH)D levels < 30 nmol/l, and also in participants aged > 65 years [8]. In addition, a 11% reduction in falls was reported in a separate recent meta-analysis of RCTs, with the effect stronger in

those with baseline 25(OH)D levels <75 nmol/l [9]. However, these results must be interpreted in light of the heterogeneity in types and dosing regimens of vitamin D interventions. The RCTs have also used a variety of different assays for assessment of serum 25(OH)D and there are well-reported method-related differences in serum 25(OH)D estimates [10], which may also be confounding some of the meta-analyses, especially when thresholds are applied. Recently, concern has been raised regarding adverse effects on risk of falling in studies using high-dose vitamin D [11].

Several studies have reported associations between different single nucleotide polymorphisms (SNPs) in the VDR and muscle strength [12; 13; 14]. So far, this has not been addressed in a RCT setting, and whether different SNPs could affect the results of vitamin D intervention on muscle strength is therefore unknown.

Quality of life could be regarded an integrative measure of self-perceived health. This should be highly relevant in the context of the possible pleiotropic effects of vitamin D, where quality of life could be considered a composite endpoint integrating various health effects of vitamin D. Observational studies have reported positive associations between serum 25(OH)D levels and quality of life [15; 16]. The results from intervention studies are, however, conflicting [17; 18; 19; 20].

In 2007-2009, we performed a vitamin D supplementation RCT in postmenopausal women with changes in bone mineral density as the primary endpoint [21]. When planning the study, we hypothesised that a higher dose of vitamin D would be more efficient than a standard dose, and we therefore compared two different doses of vitamin D_3 co-administrated with calcium. In the present work, we present the results in relation to the secondary outcomes muscle strength, balance and self-reported quality of life, which may be of importance in the on-going discussion on possible detrimental effects of high-dose vitamin D. As the participants were genotyped for SNPs in genes related to vitamin D metabolism [22], we also had the possibility to study whether vitamin D supplementation affected muscle strength differently on the basis of genotype.

Materials and methods

Study population

The study population consisted of 297 postmenopausal women aged 50-80 years with a bone mineral density T-score of ≤ -2.0 in either lumbar spine (L2.4) or total hip as measured by dual X-ray absorptometry (DEXA). The women were recruited through a number of means, including through advertisement, through other studies where bone mineral density was measured, or through the outpatient clinic at the University Hospital of North Norway, see flow chart (Fig. 1). The inclusion procedure is described in detail elsewhere [21]. Exclusion criteria were use of antiresorptive therapy or hormonal replacement therapy less then 12 months prior to the study, use of steroids, renal stone disease, coronary heart disease, cancer, diabetes, granulomatous disease, hypertension (as defined by systolic blood pressure > 175 mmHg or diastolic blood pressure > 105 mmHg), renal failure (as defined by serum creatinine > 110 µmol/l), hypercalcemia (as defined as serum calcium > 2.55 mmol/l), or suspected primary hyperparathyroidism (as defined by serum calcium > 2.50 mmol/l combined with plasma parathyroid hormone (PTH) levels > 5.0 pmol/l or serum calcium > 2.45 mmol/l combined with plasma PTH > 7.0 pmol/l.)

Protocol

The participants were screened with a questionnaire, followed by a physical examination including DEXA, and blood sampling. Those who met the inclusion criteria and signed the informed consent, were invited to a first visit where participants were requested to cease any ongoing calcium or vitamin D supplementation. All the participants then received a daily dose of 1000 mg calcium plus 800 IU vitamin D_3 (Calcigran Forte®). In addition, they were randomized to either capsules containing 20 000 IU vitamin D_3 (the high-dose group) or similar-looking placebo capsules (the standard-dose group) to be taken twice a week. This constituted an average daily dose of 6500 IU vitamin D3 in the

high-dose group and 800 IU vitamin D3 in the standard-dose group. The randomization was performed by the central randomization unit at the University Hospital of North Norway using block randomization with various block sizes, stratified by smoking (yes/no) and previous bisphosphonate use (yes/no). The randomization numbers with treatment allocation were given directly to the hospital pharmacy who prepared the medication boxes, which were then given to the study nurse at the baseline visit, ensuring blinding for the participants, the study nurses and the researchers involved in the RCT.

The intervention period lasted for 1 year with visits every 3 months on which occasions, blood sampling, registration of adverse effects and counting of returned study medication were performed. The study was approved by the Regional Committee for Medical Research Ethics, the Norwegian Data Inspectorate and the Norwegian Medicines Agency. The study was registered at ClinicalTrials.gov (NCT 00491920). The participants signed a written consent before inclusion. They could refuse the genotyping and still enter the main study, which two participants chose to do.

Measurements

Physical examinations

All the measurements were performed at the Research Unit at the University Hospital of North Norway, Tromsø, by trained research technicians. This well-established research unit had previous experience with muscle testing [23], and underwent extensive training ahead of the start of the RCT. Where possible, the same technician performed both baseline and follow-up tests in the same participant, but due to logistic reasons, this was not feasible in all cases.

Handgrip strength was measured using a Martin vigorimeter (Elmed Inc., Addison, IL, USA). For both the dominant and non-dominant hand, the best result out of three tests, with at least 15 seconds break between each test, was noted. Knee extensor strength was measured by knee

extension of the dominant leg (peak torque) using a NORM dynamometer (CSMI, Norwood, MA, USA). Each test for knee extensor strength consisted of five repetitions with submaximal extension and flexion at 90 °/second followed by one minute of rest and thereafter five maximal repetitions at 90 °/second. As the producer listed osteoporosis as a relative contraindication towards performing the test, this measurement was restricted to those with osteopenia (T-score > -2.5). Balance was assessed using tandem test, where the participants were asked to stand with their feet in three different positions as shown in figure 2, each for 10 seconds. Points were given equal to the time in seconds when the correct position was maintained (without needing support or losing the balance), resulting in a maximum score of 30 points. Body height and weight were measured without shoes and with light clothing at baseline and 12 months.

Questionnaires

The participants self-completed in the EuroQol five dimensions (EQ-5D)/three levels questionnaire, including a visual analogue score (VAS) of perceived health, at baseline and 12 months. A EQ-5D index score, based on values derived from a large population-based sample from the UK [24], was calculated according to the user manual [25]. A food-frequency questionnaire was also self-completed at baseline, and the intakes of vitamin D and calcium were calculated [26]. The International Physical Activity Questionnaire (IPAQ), (short, last 7 days, self-administered format) was also completed at baseline, and the amount of physical activity was calculated based on reported light, moderate and vigorous activities, and reported as units of metabolic equivalents (MET)-min/week, in accordance with the IPAQ guidelines. The participants were then classified according to the IPAQ groups inactive, minimally active or health-enhancing physically active [27].

Biochemical measurements

Non-fasting blood samples were taken and processed to serum, which was subsequently aliquoted and stored at -70°C for later analyses. Serum 25(OH)D was originally analysed at the Hormone Laboratory, Haukeland University Hospital, using an in-house developed liquid chromatography double mass spectrometry method [28]. The between-day precision was ≤ 8.7%. However, in order to standardize our results and make them comparable across different studies and countries, we recently re-analyzed our stored samples from baseline and 12 months using the LC-MS/MS method at the *Cork Centre for Vitamin D and Nutrition Research,* which is certified by the Centers for Disease Control and Prevention's Vitamin D Standardization-Certification Program (VDSCP)[10]. In addition to being a participant in VDSCP, the laboratory is monitored on an on-going basis by participation in the Vitamin D External Quality Assessment Scheme (DEQAS) (Charing Cross Hospital, London, UK). These standardized 25(OH)D results will be used in the present paper.

All genotyping was performed by KBioscience (http://www.kbioscience.co.uk) using KASP SNP genotyping system. KASP is a competitive allele-specific PCR incorporating a FRET quencher cassette. The SNPs were originally selected based on their reported associations with serum 25(OH)D levels [22]. Thus, the following SNPs were included in the present analyses: rs2282679 at the DBP gene, the rs10741657 at the 25-hydroxylase/CYP2R1 gene, and rs3829251 at the 7-dehydrocholesterol reductase /NAD synthetase 1 gene. In addition, the 4 most important polymorphisms in the VDR (BsmI (rs1544410), TaqI (rs731236), ApaI (rs7975232), FokI (rs2228570) were also included.

Statistical analyses

We used the statistical software package SPSS version 22 for the present analyses. Descriptive results are shown as mean (SD), unless otherwise stated. Independent t-tests or chi square tests were used for between-groups comparisons, and paired t-tests were used to assess

changes within each treatment group. Subgroup effects were studied in predefined strata of below/above median baseline serum 25(OH)D and PTH and age below/above 65 years. One-way ANOVA followed by Bonferroni correction was used for comparing muscle strength related to different genotypes. Regression analyses with an interaction term was used to assess statistical interaction between SNPs and the effect of treatment group on muscle strength. A *P* value < 0.05 was regarded as statistically significant.

Power calculations

The power calculations were based on changes in the primary endpoint bone mineral density, as published previously [21]. However, with the present sample size, the study had 80% power to detect a difference in the change in handgrip strength of 3.5 kPa between the treatment groups, given a SD of 10 kPa and an alfa value of 0.05.

Results

Adherence and side effects

Based on pill count data for those participants completing the study, the adherence was calculated to 97% for the study medication and 92% for Calcigran Forte®. Side effects were not different between the two groups, and are reported in detail elsewhere [21]. Of note, there was no difference in occurrence of urolithiasis or fractures. Falls were not specifically assessed in the present study. In relation to biochemical indices, the decrease in PTH was significantly greater, while the increments in $1,25(OH)_2D$, serum calcium and urinary calcium/creatinine ratio were significantly greater in the high-dose vitamin D group as compared to the standard-dose vitamin D group [21].

Results of the secondary outcome measures

Table 1 shows the baseline characteristics of the participants. There were no statistical significant differences between the treatment groups. Delta values during the study for each treatment group are shown in Table 2. Mean serum 25(OH)D increased significantly in both groups (P<0.01 for both), but the increment was substantially greater (P<0.01) in the high-dose group. There were no significant differences (P>0.2 for all) between the high- and standard-dose groups with respect to any of the other variables relating to muscle strength or quality of life. The change in proportion who scored less than maximum at the tandem test did not differ between the treatment groups (P=0.77). The results were similar in analyses stratified by median 25(OH)D or PTH, or age above/below 65 years (data not shown). Table 3 shows the distribution of SNPs and the association with muscle strength at baseline. Those being major homozygote (GG) at the rs3829251 in the 7-dehydrocholesterol reductase /NAD synthetase 1 gene had significantly better grip strength at the dominant (P<0.05) and non-dominant (P<0.01) hands. There was also a significant interaction between treatment group and the genotypes at this SNP (P for interaction 0.04) in relation to change in handgrip strength of the non-dominant side. Thus, in the minor homozygote group (AA), handgrip strength decreased in the high-dose vitamin D group (by -5.8 kPa), while it increased (by 7.2 kPa) in the standard-dose group, the difference being statistically significant at P=0.02 (Table 4). However, it should be noted that the group sample sizes were small. SNPs in other vitamin D-related genes did not appear to interact with the effect of vitamin D intervention on change in muscle strength (data not shown).

Discussion

In this 12-month RCT in postmenopausal women with poor bone health, there was no significant difference in muscle strength, balance or quality of life between the group randomized to the high-dose vitamin D_3 supplementation regimen (6,500 IU/day on average) and that receiving standard-dose vitamin D_3 (800 IU/day), both regimens co-administered with calcium.

The strengths of this study were the size of the study, the high completion rate and the high adherence to the study medication. The doses of vitamin D used were sufficient to provide for a substantial rise in serum 25(OH)D as confirmed by measurements made with a CDC-certified LC-MS/MS method. The outcome measures were validated, and included both upper and lower extremity strength as well as proximal and distal muscle performance. In addition, the EQ-5D questionnaire is validated in a wide range of conditions, although it should be noted that it has been subsequently refined by including five instead of three levels of answer alternatives.

Because the measurement of knee extensor strength was restricted to those with osteopenia and not osteoporosis, we had less power to assess proximal strength, and this was a limitation both in the main and subgroup analyses.

The interpretation of the results was also limited by the fact that the majority of study participants were of adequate vitamin D status even at inclusion to the study. For example, only 4 and 24% had baseline serum 25(OH)D levels < 30 and < 50 nmol/l, respectively, and 30% had baseline 25(OH)D levels > 75 nmol/l. Accordingly, these results align closely with the findings from most recently published meta-analyses on the effect of vitamin D on muscular strength, where the positive effect was significant only evident in those with baseline serum 25(OH)D levels <30 nmol/l [8]. The results from the present RCT may also support the findings from observational studies which show the inverse association between vitamin D status and muscular strength plateaus when serum 25(OH)D levels exceed 40 nmol/l [7]. In contrast, there are observational data to show that the OR for having sarcopenia in Korean women aged 50 years and older increased not only in vitamin D

deficiency (as defined as serum 25(OH)D < 37.5 nmol/l) but also in the range 37.4 – 75 nmol/l as compared to > 75 nmol/l [29]. However, ethnic differences in vitamin D metabolism as well as in associations between vitamin D status and different health outcomes have been reported [30], and thus, our results are restricted to Caucasian women only.

Of importance, there was no evidence of negative effects of high-dose as compared to standard-dose vitamin D supplementation, except for a minor increase in serum and urinary calcium of uncertain clinical importance. Unfortunately, falls were not specifically assessed in our study. In most of the clinical trials reporting detrimental effects of high-dose vitamin D on falls and fractures, vitamin D was administered by infrequent (monthly or yearly) bolus dosing [11]. It has been speculated whether such high doses upregulate the expression of 24-hydroxylase, which will increase the deactivation of the active metabolite 1,25-dihydroxyvitamin D and consequently lower its levels [11]. In addition, such a dosing regimen will lead to large fluctuations in the levels of the short-living mother compound cholecalciferol, which might affect intracellular vitamin D metabolism [31]. Clearly, this issue needs further exploration [31].

The tandem test chosen to evaluate balance in the present study turned out to be less useful in this healthy population, where the majority achieved maximum score already at baseline. Thus, this instrument did not discriminate balance sufficiently, and it is worth noting that other studies using body sway measurements have reported 28% improvement in body sway when vitamin D and calcium was given for 12 months as compared to calcium alone [32]. In a systematic review, four out of eight randomized trials using vitamin D reported improvement in body sway [33]. Furthermore, of those studies reporting an improvement, all had low serum 25(OH)D as an inclusion criteria [33].

High-dose vitamin D did supplementation not improve quality of life as compared to standard-dose. This is in accordance with other studies in elderly community-living participants [19; 20], and in heart failure patients [34]. However, other studies have reported improvement in quality of life in patients with irritable bowel syndrome [17] as well as in patients with Crohn's disease, the latter in an uncontrolled trial [18].

As previous reports have demonstrated an influence by SNPs in the VDR gene on muscle strength [12; 13; 14], we performed analyses stratified by these SNPs as well as SNPs in genes encoding central enzymes in the vitamin D metabolism pathways. There was some evidence, both in cross-sectional analyses at baseline, and in the results after intervention, that SNPs at rs3829251 affected the relationship between vitamin D and muscle strength. These SNPs are contained within the gene coding for 7-dehydrocholesterol reductase, an enzyme central in the UVB-induced production of previtamin D in the skin. Thus, those being major homozygote had a greater grip strength than those being heterozygote or minor homozygote, and within the minor homozygote subgroup, muscle strength increased in the standard-dose vitamin D group, while it decreased in the high-dose vitamin D group. This was not consistent at all muscle measurement sites, the groups were of small sample size, and the analyses were not adjusted for multiple comparisons. In light of multiple testing, our results could therefore represent random findings, and thus would need to be confirmed in other studies. We are not aware of any previous studies analysing this SNP in relation to muscle strength.

Interestingly, we could not reproduce the previously reported associations between SNPs in the VDR and muscle strength. It should be noted that the groups with minor homozygotes in general were of small sample size, and type II errors can therefore not be excluded. Also, the high baseline vitamin D status might have obscured possible effects of genetic polymorphisms.

This study was designed to compare two doses of supplemental vitamin D, and not to assess the effect of vitamin D itself. The results of this study do not provide support for using high-dose vitamin D supplements in already vitamin D-replete subjects in terms of improving muscular

strength, balance, or quality of life. There were, however, no indications of harmful clinical effects, although a minor increase in serum and urinary calcium was noted, and the safety assessment was limited by the fact that falls were not specifically assessed. Clearly, the safety of long-term use of high-dose vitamin D could not be assessed from this one-year trial. Future studies should include vitamin D deficient participants only, and fall assessment should be included as an outcome measure in all high-dose vitamin D trials.

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References

1. DeLuca, H. F. (2004). Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr* **80**, 1689s-96s.
2. Bikle, D. D. (2016). Extraskeletal actions of vitamin D. *Ann N Y Acad Sci* **1376**, 29-52.
3. Jorde, R. & Grimnes, G. (2015). Vitamin D and health: the need for more randomized controlled trials. *J Steroid Biochem Mol Biol* **148**, 269-74.
4. Girgis, C. M., Mokbel, N., Cha, K. M., Houweling, P. J., Abboud, M., Fraser, D. R., Mason, R. S., Clifton-Bligh, R. J. & Gunton, J. E. (2014). The vitamin D receptor (VDR) is expressed in skeletal muscle of male mice and modulates 25-hydroxyvitamin D (25OHD) uptake in myofibers. *Endocrinology* **155**, 3227-37.

5. Bischoff-Ferrari, H. A., Borchers, M., Gudat, F., Durmuller, U., Stahelin, H. B. & Dick, W. (2004). Vitamin D receptor expression in human muscle tissue decreases with age. *J Bone Miner Res* **19**, 265-9.

6. Wicherts, I. S., van Schoor, N. M., Boeke, A. J., Visser, M., Deeg, D. J., Smit, J., Knol, D. L. & Lips, P. (2007). Vitamin D status predicts physical performance and its decline in older persons. *J Clin Endocrinol Metab* **92**, 2058-65.

7. Bischoff-Ferrari, H. A., Dietrich, T., Orav, E. J., Hu, F. B., Zhang, Y., Karlson, E. W. & Dawson-Hughes, B. (2004). Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged > or =60 y. *Am J Clin Nutr* **80**, 752-8.

8. Beaudart, C., Buckinx, F., Rabenda, V., Gillain, S., Cavalier, E., Slomian, J., Petermans, J., Reginster, J. Y. & Bruyere, O. (2014). The effects of vitamin D on skeletal muscle strength, muscle mass, and muscle power: a systematic review and meta-analysis of randomized controlled trials. *J Clin Endocrinol Metab* **99**, 4336-45.

9. LeBlanc, E. S. & Chou, R. (2015). Vitamin D and falls-fitting new data with current guidelines. *JAMA Intern Med* **175**, 712-3.

10. Cashman, K. D., Dowling, K. G., Skrabakova, Z., Gonzalez-Gross, M., Valtuena, J., De Henauw, S., Moreno, L., Damsgaard, C. T., Michaelsen, K. F., Molgaard, C., Jorde, R., Grimnes, G., Moschonis, G., Mavrogianni, C., Manios, Y., Thamm, M., Mensink, G. B., Rabenberg, M., Busch, M. A., Cox, L., Meadows, S., Goldberg, G., Prentice, A., Dekker, J. M., Nijpels, G., Pilz, S., Swart, K. M., van Schoor, N. M., Lips, P., Eiriksdottir, G., Gudnason, V., Cotch, M. F., Koskinen, S., Lamberg-Allardt, C., Durazo-Arvizu, R. A., Sempos, C. T. & Kiely, M. (2016). Vitamin D deficiency in Europe: pandemic? *Am J Clin Nutr* **103**, 1033-44.

11. Sanders, K. M. & Seibel, M. J. (2016). New findings on vitamin D3 supplementation and falls - when more is perhaps not better. *Nat Rev Endocrinol* **12**, 190-1.

12. Hopkinson, N. S., Li, K. W., Kehoe, A., Humphries, S. E., Roughton, M., Moxham, J., Montgomery, H. & Polkey, M. I. (2008). Vitamin D receptor genotypes influence quadriceps strength in chronic obstructive pulmonary disease. *Am J Clin Nutr* **87**, 385-90.

13. Bozsodi, A., Boja, S., Szilagyi, A., Somhegyi, A., Varga, P. P. & Lazary, A. (2016). Muscle strength is associated with vitamin D receptor gene variants. *J Orthop Res*.

14. Walsh, S., Ludlow, A. T., Metter, E. J., Ferrucci, L. & Roth, S. M. (2016). Replication study of the vitamin D receptor (VDR) genotype association with skeletal muscle traits and sarcopenia. *Aging Clin Exp Res* **28**, 435-42.

15. Rafiq, R., Swart, K. M., van Schoor, N. M., Deeg, D. J., Lips, P. & de Jongh, R. T. (2014). Associations of serum 25-hydroxyvitamin D concentrations with quality of life and self-rated health in an older population. *J Clin Endocrinol Metab* **99**, 3136-43.

16. Jelinek, G. A., Marck, C. H., Weiland, T. J., Pereira, N., van der Meer, D. M. & Hadgkiss, E. J. (2015). Latitude, sun exposure and vitamin D supplementation: associations with quality of

life and disease outcomes in a large international cohort of people with multiple sclerosis. *BMC Neurol* **15**, 132.

17. Abbasnezhad, A., Amani, R., Hajiani, E., Alavinejad, P., Cheraghian, B. & Ghadiri, A. (2016). Effect of vitamin D on gastrointestinal symptoms and health-related quality of life in irritable bowel syndrome patients: a randomized double-blind clinical trial. *Neurogastroenterol Motil*.

18. Yang, L., Weaver, V., Smith, J. P., Bingaman, S., Hartman, T. J. & Cantorna, M. T. (2013). Therapeutic effect of vitamin d supplementation in a pilot study of Crohn's patients. *Clin Transl Gastroenterol* **4**, e33.

19. Patil, R., Karinkanta, S., Tokola, K., Kannus, P., Sievanen, H. & Uusi-Rasi, K. (2015). Effects of Vitamin D and Exercise on the Wellbeing of Older Community-Dwelling Women: A Randomized Controlled Trial. *Gerontology*.

20. Sanders, K. M., Stuart, A. L., Williamson, E. J., Jacka, F. N., Dodd, S., Nicholson, G. & Berk, M. (2011). Annual high-dose vitamin D3 and mental well-being: randomised controlled trial. *Br J Psychiatry* **198**, 357-64.

21. Grimnes, G., Joakimsen, R., Figenschau, Y., Torjesen, P. A., Almas, B. & Jorde, R. (2012). The effect of high-dose vitamin D on bone mineral density and bone turnover markers in postmenopausal women with low bone mass--a randomized controlled 1-year trial. *Osteoporos Int* **23**, 201-11.

22. Didriksen, A., Grimnes, G., Hutchinson, M. S., Kjaergaard, M., Svartberg, J., Joakimsen, R. M. & Jorde, R. (2013). The serum 25-hydroxyvitamin D response to vitamin D supplementation is related to genetic factors, BMI, and baseline levels. *Eur J Endocrinol* **169**, 559-67.

23. Svartberg, J., Agledahl, I., Figenschau, Y., Sildnes, T., Waterloo, K. & Jorde, R. (2008). Testosterone treatment in elderly men with subnormal testosterone levels improves body composition and BMD in the hip. *Int J Impot Res* **20**, 378-87.

24. Dolan, P. (1997). Modeling valuations for EuroQol health states. *Med Care* **35**, 1095-108.

25. Brooks, R. (1996). EuroQol: the current state of play. *Health Policy* **37**, 53-72.

26. Andersen, L. F., Solvoll, K., Johansson, L. R., Salminen, I., Aro, A. & Drevon, C. A. (1999). Evaluation of a food frequency questionnaire with weighed records, fatty acids, and alpha-tocopherol in adipose tissue and serum. *Am J Epidemiol* **150**, 75-87.

27. Craig, C. L., Marshall, A. L., Sjostrom, M., Bauman, A. E., Booth, M. L., Ainsworth, B. E., Pratt, M., Ekelund, U., Yngve, A., Sallis, J. F. & Oja, P. (2003). International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc* **35**, 1381-95.

28. Grimnes, G., Almaas, B., Eggen, A. E., Emaus, N., Figenschau, Y., Hopstock, L. A., Hutchinson, M. S., Methlie, P., Mihailova, A., Sneve, M., Torjesen, P., Wilsgaard, T. & Jorde, R. (2010). Effect of smoking on the serum levels of 25-hydroxyvitamin D depends on the assay employed. *Eur J Endocrinol* **163**, 339-48.

29. Park, S., Ham, J. O. & Lee, B. K. (2014). A positive association of vitamin D deficiency and sarcopenia in 50 year old women, but not men. *Clin Nutr* **33**, 900-5.

30. Freedman, B. I. & Register, T. C. (2012). Effect of race and genetics on vitamin D metabolism, bone and vascular health. *Nat Rev Nephrol* **8**, 459-66.

31. Hollis, B. W. & Wagner, C. L. (2013). Clinical review: The role of the parent compound vitamin D with respect to metabolism and function: Why clinical dose intervals can affect clinical outcomes. *J Clin Endocrinol Metab* **98**, 4619-28.

32. Pfeifer, M., Begerow, B., Minne, H. W., Suppan, K., Fahrleitner-Pammer, A. & Dobnig, H. (2009). Effects of a long-term vitamin D and calcium supplementation on falls and parameters of muscle function in community-dwelling older individuals. *Osteoporos Int* **20**, 315-22.

33. Rejnmark, L. (2011). Effects of vitamin d on muscle function and performance: a review of evidence from randomized controlled trials. *Ther Adv Chronic Dis* **2**, 25-37.

34. Witham, M. D., Crighton, L. J., Gillespie, N. D., Struthers, A. D. & McMurdo, M. E. (2010). The effects of vitamin D supplementation on physical function and quality of life in older patients with heart failure: a randomized controlled trial. *Circ Heart Fail* **3**, 195-201.

Legends to Figures

Fig. 1. Flow chart of study participants

Fig 2. Tandem test

Table 1. Baseline characteristics

	High dose vitamin D (n=149)	Standard dose vitamin D (n=148)
Age (years)	62.8±7.4	63.4±6.9
Age since menopause (years)	14.0±8.5	14.8±8.9
BMI (kg/cm^2)	24.9±3.4	24.6±3.3
Serum 25(OH)D (nmol/l)	64.7±21.4	64.1±20.0
Estimated vitamin D intake (mcg/day)	9.1±6.3	8.2±6.1
Estimated calcium intake (mg/day)	797±313	823±335
MET-min/week	3165±3207	3205±3504
Physical activity (IPAQ)		
Inactive	21.7	24.8
Minimally active	41.9	41.9
Health enhancing physically active	36.4	33.3
Proportion smokers (%)	22.2	24.3
Proportion with osteoporosis (%)	58	47
Grip strength dominant hand (kPa)	54.9±16.4	53.5±14.4
Grip strength non-dominant hand (kPa)	51.8±17.7	51.3±15.7
Knee extensor strength (Nm)	72.7±16.2	70.8±15.7
Tandem test, proportion scoring less than full score (%)	9	13
VAS of perceived health	81.4±13.0	81.4±14.0
EQ-5D index	0.85±0.18	0.86±0.14
EQ-5D - Mobility (%)		
No problem walking	86.7	83.6

Some problems walking	13.3	16.4
Confined to bed	0	0
EQ-5D - Self-Care (%)		
No problems with self-care	97.8	100
Some problems with self-care	2.2	0
Unable to wash or dress	0	0
EQ-5D - Usual activities (%)		
No problems	88.1	86.4
Some problems	11.9	13.6
Unable to perform usual activities	0	0
EQ-5D - Pain/Discomfort (%)		
No pain or discomfort	51.1	50.0
Moderate pain or discomfort	46.7	49.3
Extreme pain or discomfort	2.2	0.7
EQ-5D - Anxiety/Depression (%)		
Not anxious or depressed	83.0	80.7
Moderately anxious or depressed	17.0	19.3
Extremely anxious or depressed	0	0

BMI, body mass index; EQ-5D, Euroqual 5 dimensions; MET, metabolic equivalents; VAS visual analogue scale; 25(OH)D, 25-hydroxyvitamin D

Table 2. Change in serum 25(OH)D, muscle strength and quality of life after intervention with standard or high dose vitamin D and calcium

	High dose vitamin D	Standard dose vitamin D	*P* for difference between groups
△ Serum 25(OH)D (nmol/l)	99.4±31.9[a]	17.7±16.9[a]	<0.01
△Grip strength dominant hand (kPa)	0.1±9.4	0.7±8.0	0.59
△Grip strength non-dominant hand (kPa)	1.4±9.7	1.5±10.0	0.94
△Knee extensor strength (Nm)[b]	-1.4±7.6	0.5±8.0	0.24
△VAS	-1.5±12.8	-1.8±12.1	0.84
△EQ-5D index	-0.02±0.16	-0.02±0.17	0.85

△ change from 12-months minus baseline value; EQ-5D, Euroqual 5 dimensions; VAS visual analogue scale; 25(OH)D, 25-hydroxyvitamin D

[a]change from baseline, p<0.01

[b] available measurements in 46 in the high dose and 59 in standard dose group

Table 3. Distribution of SNPs and correlation with muscle strength

SNP	genotype	n	Grip strength dominant side (kPa)	*P*	n	Grip strength non-dominant side (kPa)	*P*	n	Knee extensor strength (Nm)	*P*
Rs2282679	AA	164	53.6 (51.5-55.6)	0.83	162	51.0 (48.5-53.5)	0.76	78	73.0 (69.3-76.7)	0.17
	AC	113	54.7 (51.7-57.8)		112	51.9 (48.9-54.9)		36	66.9 (62.2-71.5)	
	CC	13	54.0 (38.8-69.2)		13	54.2 (40.5-67.8)		7	70.9 (54.2-87.5)	
Rs10741657	GG	93	54.5 (51.7-57.3)	0.58	92	52.0 (48.5-55.4)	0.62	45	71.9 (67.4-76.5)	0.52
	GA	144	53.1 (50.4-55.8)		144	50.5 (47.8-53.3)		57	69.4 (64.7-74.1)	
	AA	55	55.4 (51.5-59.4)		53	52.9 (48.9-57.0)		19	73.8 (68.1-79.5)	

Rs3829251	GG	166	56.0 (53.6-58.3)	<0.05	165	54.2 (51.8-56.7)	<0.01*	64	73.0 (69.4-76.6)	0.35
	GA	98	51.5 (48.4-54.7)		96	47.1 (43.7-50.6)		41	69.1 (63.3-75.0)	
	AA	27	50.4 (45.6-55.3)		27	49.4 (44.1-54.7)		16	68.1 (60.7-75.6)	
Rs1544410	GG	98	54.8 (51.7-57.9)	0.48	96	52.3 (48.8-55.8)	0.24	45	72.4 (67.6-77.1)	0.60
	GA	139	52.8 (50.3-55.3)		139	49.8 (47.1-52.5)		51	69.3 (64.5-74.2)	
	AA	53	55.2 (50.8-59.5)		53	54.0 (49.9-58.1)		25	72.2 (66.8-77.5)	
Rs731236	TT	94	53.9 (50.9-56.8)	0.73	92	51.7 (48.1-55.2)	0.29	44	71.7 (67.0-76.4)	0.82
	TC	136	53.2 (50.7-55.8)		135	49.9 (47.1-52.6)		50	70.1 (65.4-74.8)	
	CC	53	55.1 (50.8-59.4)		53	53.9 (49.8-58.0)		25	72.2 (66.8-77.5)	
Rs7975232	AA	104	53.5 (50.4-56.5)	0.07	103	51.6 (48.4-54.8)	0.19	41	71.6 (67.0-76.2)	0.13
	CA	133	52.6 (50.2-54.9)		132	49.8 (47.0-52.5)		52	68.1 (63.4-72.8)	

	CC	53	58.3 (53.5-63.1)		52	54.6 (50.0-59.2)		28	75.6 (69.7-81.5)	
Rs2228570	CC	109	53.0 (50.0-55.9)	0.39	107	50.5 (47.4-53.5)	0.44	46	70.4 (66.4-74.5)	0.44
	TC	139	55.3 (52.9-57.7)		139	52.7 (49.9-55.4)		56	70.1 (65.4-74.8)	
	TT	41	52.6 (47.0-58.2)		40	49.7 (44.3-55.0)		19	75.3 (67.6-83.0)	

*Statistically significant different between GG and GA also after Bonferroni correction

Table 4. Change in muscle strength after intervention with standard or high dose vitamin D and calcium stratified by genotypes in rs3829251

		Hand grip dominant side		Hand grip non-dominant side		Knee extensor strength	
		n	change (kPa)	n	change (kPa)	n	change (Nm)
GG	High dose vitamin D	72	0.8	71	1.6	24	-2.4
	Standard dose vitamin D	82	0.3	83	0.8	31	-0.4
GA	High dose vitamin D	50	0.1	48	2.8	19	-1.1
	Standard dose vitamin D	39	-0.3	38	0.5	16	2.3
AA	High dose vitamin D	10	-4.5	10	-5.8 *	3	5.3
	Standard dose vitamin D	15	3.1	15	7.2	10	-1.5

* *P* for difference <0.05