Effects of vitamin D supplementation on neuroplasticity in older adults: A double-blinded, placebo controlled randomised trial

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Running Title: Neural adaptations to vitamin D

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ABSTRACT

Purpose: To examine the effects of vitamin D supplementation on neuroplasticity, serum brain-derived neurotrophic factor (BDNF), muscle strength and function in older adults.

Methods: This was a 10-week double-blinded, placebo controlled randomised trial in which 26 older adults with 25-hydroxyvitamin D [25OHD] concentrations 25-60 nmol/L were randomised to 2000IU/d vitamin D₃ or matched placebo. Single and paired-pulse transcranial magnetic stimulation applied over the motor cortex was used to assess changes in motor evoked potentials (MEPs) and short-interval intracortical inhibition (SICI), as measures of corticospinal excitability and inhibition respectively, by recording EMG responses to stimulation from the wrist extensors. Changes in muscle strength, stair climbing power, gait (timed-up-and-go), dynamic balance (four square step test), serum 25(OH)D and BDNF concentrations were also measured.

Results: After 10-weeks, mean 25(OH)D levels increased from 46 to 81 nmol/L in the vitamin D group with no change in the placebo group. The vitamin D group experienced a significant 8-11% increase in muscle strength and a reduction in cortical excitability (MEP amplitude) and SICI relative to baseline (all $P < 0.05$), but these changes were not significantly different from placebo. There was no effect of vitamin D on muscle power, function or BDNF.

Conclusions: Daily supplementation with 2000IU vitamin D₃ for 10-weeks had no significant effect on neuroplasticity compared to placebo, but the finding that vitamin D treatment alone was associated with a decrease in corticospinal excitability and intracortical inhibition warrants further investigation as this suggests that it may improve the efficacy of neural transmission within the corticospinal pathway.

Keywords: vitamin D, neuroplasticity, muscle strength, aging, nutrition

MINI-ABSTRACT

Vitamin D can improve muscle function and reduce falls, but whether it can strengthen neural connections within the brain and nervous system is not known. This 10-week randomised controlled trial indicates that treatment with 2000IU/d vitamin D₃ does not significantly alter neuroplasticity relative to placebo in older adults.

INTRODUCTION

The benefits of vitamin D on muscle function and falls risk in older adults [1, 2] is supported by evidence that vitamin D receptors (VDR) are located in skeletal muscle [3], VDR activation may promote muscle protein synthesis [4] and the absence of VDRs in knockout mice is associated with muscle fibre atrophy [5]. VDRs are also located in the brain, including the primary motor cortex which is a region responsible for controlling movement [6, 7]. The central nervous system (CNS) also has the ability to activate the biological active form of vitamin D (1,25-dihydroxyvitamin D) [6, 7]. Since the role of the CNS is to activate muscles to produce co-ordinated contractions and an appropriate motor response, the effects of vitamin D on muscle function may also be mediated by changes in neuroplasticity, or the ability of the CNS to adapt and strengthen neural connections.

Transcranial magnetic stimulation (TMS) is a non-invasive technique that can quantify the strength of neural projections from the brain to the muscles. When single-pulse TMS is applied to the motor cortex at an appropriate stimulation intensity, it induces a series of action potentials in the corticospinal tract resulting in a muscle response that can be recorded as a motor-evoked potential (MEP) [8, 9]. Changes in MEP amplitude are thought to represent adjustments in the synaptic efficacy of corticospinal neurons onto the motoneuron pool innervating the target muscle. Using a paired-pulse TMS protocol, it is also possible to measure intracortical inhibition (ICI) confined to the motor cortex [9]. In this instance, a sub-threshold conditioning stimulus precedes a supra-threshold test stimulus, which results in a suppressed MEP relative to a single-pulse stimuli at the same intensity. This conditioning stimulus activates low-threshold inhibitory circuits that use the neurotransmitter gamma-aminobutyric acid (GABA), resulting in synaptic inhibition of corticospinal neurons [10]. Thus, a reduction in ICI represents a down-regulation of inhibitory cortical processes, which

implies that there is a decrease in the responsiveness of the intracortical neurons or a reduction in the synaptic efficacy between intracortical and corticospinal neurons. A decrease in ICI has also been reported to enhance the efficiency of neural transmission along the corticospinal tract and improve subsequent excitatory drive to produce the desired movement.

Previous research shows that neurotrophins such as brain-derived neurotrophic factor (BDNF), play a crucial role in the induction of neuroplasticity [11]. BDNF is expressed throughout the CNS and has been shown to stimulate the development and differentiation of new neurons, promote neuron survival and differentially modulate neuroplasticity in response to various interventions, such as motor learning [12-14]. However, no previous studies have investigated whether vitamin D supplementation can alter circulating BDNF concentrations.

The aim of this 10-week double-blinded, placebo controlled randomised trial was to investigate the effects of vitamin D supplementation on corticospinal excitability and intracortical inhibition in older adults with insufficient serum 25(OH)D levels. Secondary aims of the study were to investigate the effects of vitamin D on serum BDNF concentrations, and muscle strength and functional performance.

MATERIALS AND METHODS

Participants

Men and women aged ≥ 60 years with serum 25(OH)D concentrations 25-60 nmol/L were recruited from the local community in Melbourne, Australia. Participants were excluded for the following reasons: current/prior participation in resistance training (≥ 1 week) and/or >150 minutes/week of physical activity in the last 3 months; acute/terminal illness or ongoing/unstable cardiovascular, respiratory, neurological or musculoskeletal diseases that

might limit testing; recent fracture (last 3 months); visual impairment not corrected by glasses; and currently or previously (last 2 months) taken vitamin D supplements (>200 IU/d). A total of 132 adults were pre-screened over the telephone, from which 52 were invited to have their vitamin D status assessed. Of these, 26 had a serum 25(OH)D concentration between 25 and 60 nmol/L, and were included in the study. The main reasons for exclusion were: currently taking vitamin D supplements (n=70), use of glucocorticoid (n=3) or thyroxine (n=5), 25(OH)D concentration outside the required range (n=12) or inserted titanium plates (n=5). A study flow diagram is provided in Figure 1. The trial was approved by Deakin University Human Research Ethics Committee (DUHREC 2012.051) and registered with the Australian New Zealand Clinical Trials Registry (ACTRN 12612000592842).

Study Design

This was a 10-week, double-blinded, placebo-controlled randomised trial in which 26 adults were randomized to receive 2000 IU/d vitamin D₃ (Blackmores) (n=13) or a matched placebo supplement (n=13). Participants were randomised by an independent researcher using a random number generator. All supplements were dispensed and labelled by an independent person, and participants received monthly phone calls to optimise adherence. Both study participants and research staff were blinded to the treatment code.

Measurements

All measurements were conducted at baseline and after 10-weeks.

Anthropometry

Height (cm) was measured using a Holtain wall stadiometer to the nearest mm. Weight (kg) was measured using an A&D UC-321 Series electronic scale to the nearest gram. BMI was calculated as weight (kg) divided by height in meters squared.

Electromyography (EMG)

Surface electromyography (sEMG) activity was recorded from the right extensor carpi radialis (ECR) muscle using bipolar Ag-AgCl electrodes, with an inter-electrode distance of 2-cm with a muscle belly-tendon montage. A grounding strap placed around the wrist was used as a common reference for all electrodes. All cables were fastened with tape to prevent movement artefacts. The area of electrode placement was shaved, rubbed with an abrasive skin rasp, and then cleaned with alcohol. An impedance meter was used to ensure impedance did not exceed 10k Ω prior to testing. sEMG signals were amplified (x1000), bandpass filtered (high pass at 13Hz, low pass at 1000Hz), digitized online at 2kHz for 500ms, recorded and analyzed using PowerLab 4/35 [9].

Transcranial magnetic stimulation (TMS)

Single and paired-pulse TMS was applied over the left motor cortex using a BiStim unit attached to two Magstim 200² stimulators (Magstim Co, Dyfed, UK) to produce MEPs in the right ECR. As reported previously [9], a 70-mm figure-eight coil, with an external loop diameter of 9-cm, was held tangential to the skull over the left motor cortex at the optimum scalp position ('hot spot') to elicit the largest MEP amplitude of the right ECR. The same researcher performed all measurements and participants wore a tight-fitted cap marked with a latitude-longitude co-ordinate system to locate the specific site on the skull where the coil was placed that evoked the maximum MEP amplitude. All TMS measures were taken during weak

voluntary contractions. Root mean square (rms) EMG (rmsEMG) of the ECR was obtained prior to each TMS stimulus to ensure that there were no changes in pre-stimulus rmsEMG which may have altered the MEP amplitude. Active motor threshold (AMT) was determined as the minimum stimulus intensity that produced a small MEP (200 μ V in 5 out of 10 consecutive trials) during isometric contraction of the ECR at $5\pm 2\%$ of maximal rmsEMG activity. The stimulus intensity started at 50% maximum stimulator output (MSO) and was altered in increments of $\pm 1\%$ of MSO until the appropriate threshold level was achieved. Single-pulse recruitment curves were collected during low level isometric contractions of the wrist extensors. For a single recruitment curve, 10 stimuli were delivered at each intensity from below AMT (5% stimulator output steps) to 45% above AMT. The following measures were derived from the recruitment curves: *MEP_{Max} or peak plateau*, which provides an estimate of the proportion of motoneuron pool that was activated by TMS; *slope steepness*, which provides a measure of the neurophysiological strength of intracortical and corticospinal connections, and *half peak slope (V_{50})*, which is a measure of the threshold characteristics of corticospinal cells and alpha motoneurons.

Short-interval intracortical inhibition (SICI) was assessed using paired-pulse TMS that consisted of a subthreshold conditioning stimulus that preceded a suprathreshold test stimulus [9]. The protocol for SICI included 10 unconditioned stimuli (single-pulse), with a test intensity set at 120% AMT and 10 conditioned stimuli, with a conditioning stimulus intensity set at 80% AMT. A 3 ms inter-stimulus interval between the conditioning and test stimulus was used [15]. Single and paired-pulse stimuli were presented according to a predetermined randomization protocol, with a 6-9 second period between each stimulus.

Direct muscle responses were obtained from the ECR by supramaximal electrical stimulation (pulse width 200 μ s) of the brachial plexus (Erb's point) under resting conditions (DS7A, Digitimer, UK). The site of stimulation that produced the largest M-wave was located by positioning the bipolar electrodes in the supraclavicular fossa. An increase in current strength was applied to the brachial plexus until there was no further increase observed in the amplitude of the sEMG response (M_{MAX}). To ensure maximal responses, the current was increased an additional 20% and the average M_{MAX} was obtained from five stimuli, with a period of 6-9 seconds separating each stimulus.

TMS data analysis

The peak-to-peak amplitude of MEPs evoked as a result of stimulation was measured in the ECR muscle contralateral to the cortex being stimulated in the period 10-50 ms after stimulation. MEP amplitudes were analyzed using LabChart 7.3.6 software (ADInstruments, Bella Vista, NSW, Australia) after each stimulus was automatically flagged with a cursor, providing peak-to-peak values in μ V which were then normalized to M_{MAX} [9]. To construct recruitment curves, stimulus intensity was plotted against MEP amplitude and then fitted with a non-linear Boltzmann equation [9]:

$$MEP(s) = Bottom + (Top - Bottom) / (1 + \exp^{((V_{50} - s) / slope)})$$

where s represents the stimulus intensity, Top represents the MEP plateau value, $Bottom$ represents the minimum MEP value, V_{50} represents the stimulus intensity at which MEP amplitude is halfway between top and bottom, and $slope$ represents the steepness of the curve. SICI was calculated using the following equation:

$$SICI = SP - PP / M_{MAX} \times 100$$

where SP represents the average MEP amplitude from the single-pulse stimuli and PP represents the average MEP amplitude from the paired-pulse stimuli. This equation has a direct relation to the traditional measure of SICI which is reported as a SICI ratio [9], with a decrease in inhibition representing a decrease in the numerical value.

Muscle strength

Knee extensor muscle strength was assessed on the dominant limb using the Biodex system 4 Pro isokinetic dynamometer (Biodex Medical Systems, USA). All participants were instructed to perform three maximal isokinetic leg extensions at 120, 180 and 240 degrees per second (deg/s). The highest peak knee extension torque of the three trials was taken and recorded as the maximal peak force adjusted for body weight.

Physical function

Functional stair climbing muscle power was assessed by asking participants to climb a flight of stairs (10 steps, 7.8 cm rise/step) as quickly as possible without the use of handrails or any other aid. Stair climbing power (SCP) (Watts, W) was calculated according to the method described by Lazowski et al [16]. Dynamic balance and gait was assessed using the following battery of validated tests: the Four Square Step Test and the Timed-Up-and-Go Test with a secondary cognitive task (counting backwards from 100 by 3's).

Serum 25-hydroxyvitamin D and brain-derived neurotrophic factor (BDNF)

Serum 25(OH)D was assessed from fasting, resting morning blood samples using the Liaison®25OH vitamin D TOTAL assay (Liaison®25OHD) (DiaSorin Inc., Stillwater, MN, USA). Serum BDNF concentrations were quantitatively determined by ELISA using the DuoSet ELISA Development Kit from R&D systems (Minneapolis, MN, USA).

Medical History, Diet and Physical Activity

Health and medical status, medication use, smoking status, alcohol intake and use of supplements were assessed by questionnaire. Nutrient intake was assessed using a 24-hour food recall questionnaire and analysed using the Australian specific Foodworks dietary analysis program (Xyris Software, Highgate Hill, Australia). Habitual physical activity was assessed using the CHAMPS questionnaire [17]. Adherence to the supplements were analysed by counting capsules remaining at the final testing.

Statistical and Data Analysis

Statistical analyses were conducted using SPSS for Windows (version 20, SPSS Inc, Chicago, IL). All data were screened for normality and the following variables were log transformed: serum BDNF, muscle strength, physical activity and SCP. Baseline and demographic data were assessed using independent t-tests for continuous variables and chi-square tests for categorical variables. Repeated measures ANCOVA were used to assess between group differences for the change in all TMS and muscle measures, diet, physical activity and the hormonal variables, adjusting for baseline age and gender. Paired t-tests were used to assess within group changes. Percentage changes in log-transformed serum BDNF, muscle strength and SCP represent the absolute differences from baseline multiplied by 100. Since this study was an exploratory trial, it was difficult to conduct a formal sample size analysis on the TMS outcome measures. However, using previous data from pharmacological experiments in adults,[18] we estimated that 12 participants in each group would provide at least 80% power (two-tailed, $P < 0.05$) to detect a 15% difference in mean MEP amplitude (SD of 10%) between the groups, assuming a 10% loss to follow-up.

RESULTS

Baseline characteristics

There were no significant differences between the groups for any of the baseline characteristics, with the exception that the vitamin D group were younger and had a higher dietary vitamin D intake (both $P < 0.05$) (Table 1-2).

Study attrition and adherence to supplements

One (3.8%) participant from the placebo group withdrew from study due to medical reasons (Figure 1). Mean compliance to the supplements was similar for the vitamin D (97.8%) and placebo group (88.6%).

Diet and physical activity

Diet and physical activity did not change or differ between groups throughout the study (data not shown).

Muscle strength and function

After 10-weeks, there was a significant 8-11% increase in all muscle strength measures in the vitamin D group (P ranging < 0.05 - < 0.001), with the exception of KE 120°/s (Table 2), and a 6% increase in KE 120°/s in the placebo group ($P < 0.05$). However, there were no significant between group differences for the changes in any measure of strength. Similarly, there were no within group changes or between group differences for SCP, FSST or TUG (Table 2).

TMS outcomes

The mean TMS stimulus output required to elicit AMT for the ECR muscle was no different between the groups at baseline, and did not change in either group following the intervention (Figure 2). There were also no significant between group differences for the change in any of

the corticospinal responses (group-by-time interaction: AMT% $P=0.23$; MEP_{Max}, $P=0.44$; SICI, $P=0.23$; MEP amplitude 120% AMT, $P=0.14$) (Figure 2). However, in the vitamin D group, there was a significant reduction relative to baseline in MEP_{max} [mean absolute change -5.41 (95% CI: -9.93, -0.89), $P<0.05$], MEP amplitude at 120% AMT [-5.4 (95% CI: -7.72, -2.24), $P<0.01$] and SICI [-4.00 (95% CI: -7.04, -0.96), $P<0.05$].

As shown in Figure 3, MEP amplitude decreased significantly in the majority of stimulus intensities after 10-weeks of vitamin D treatment ($P<0.05$), with no changes observed in the placebo group. However, these changes did not differ significantly between the groups. Similarly, there were no within group changes or between group differences for the changes over 10-weeks in peak plateau, V₅₀ or peak slope [group-by-time interaction: peak plateau $P=0.42$; V₅₀ $P=0.90$; slope steepness, $P=0.25$], with the exception that there was a significant increase in V₅₀ in the vitamin D group relative to baseline ($P<0.05$).

Hormonal measures

On average, serum 25(OH)D levels increased by 34 nmol/L (95% CI: 26.3, 42.2) in the vitamin D group with no change in the placebo group [-1.2 nmol/L (95% CI: -5.8, 3.4)]. There were no changes in serum calcium in either group. Serum BDNF were no different between the groups at baseline (Table 1) and did not change over time in either the vitamin D [mean percentage change (95% CI): 4.3 (-18.9, 27.6)] or placebo group [-0.1 (-33.2, 31.7)].

DISCUSSION

The main finding from this 10-week intervention in older adults with insufficient serum 25(OH)D levels was that there were no significant differences for the change in any neural or muscle measure or circulating BDNF concentrations between the vitamin D and placebo

group. While this suggests that short-term treatment with 2000 IU/d vitamin D has no marked effect on neuroplasticity in older adults, the observation that muscle strength increased and both corticospinal excitability and intracortical inhibition decreased following vitamin D treatment warrant further study as this suggests that vitamin D may improve the efficacy of neural transmission within the corticospinal pathway, and thereby facilitate motor output to improve muscle strength.

Previous research has shown that VDRs are located in most areas of the brain, especially the hippocampus and hypothalamus, as well as in cortical, subcortical and spinal motor areas which includes the motor cortex [6, 7]. The enzyme 1, α -hydroxylase, which is the rate limiting enzyme in the synthesis of vitamin D, has also been shown to be present in the CNS [7], and the influence of 1,25(OH)D on the CNS is reported to be associated with changes in the production and release of neurotrophic factors [19]. On this basis, we hypothesised that vitamin D treatment would enhance corticospinal excitability. A study in rodents reported that vitamin D treatment increased axogenesis and axon diameter resulting in higher functional recovery [20]. Similarly, vitamin D supplementation enhanced motoneuron excitability in rats as indicated by an increase in the Hoffman (H)-reflex, which is the monologue of the monosynaptic stretch reflex used to assess the excitability of the spinal alpha-motoneurons [21, 22]. There is also evidence that vitamin D acts on the peripheral nervous system, with a reduction in nerve conduction velocity associated with vitamin D deficiency [23]. In our study, the finding that vitamin D was associated with a reduction in MEP amplitude implies that there was a decrease in the physiological strength of corticospinal cell projections onto the motoneuron pool innervating the target muscle. Although we are unable to determine the underlying mechanism(s) responsible for this decrease in corticospinal excitability, it may be due to fewer motoneurons being activated along the corticospinal tract, reduced synaptic

efficacy between corticospinal cells and motoneurons or changes in the intrinsic properties of the motoneurons themselves.

It is widely established that corticospinal projections from the motor cortex are modulated by both intracortical inhibitory and excitatory circuits. Using paired-pulse TMS, we were able to quantify changes in ICI confined to the motor cortex. ICI is mediated by GABA inhibitory interneurons which selectively attenuate the activity of other neurons (pyramidal neurons) in the motor cortex, thereby affecting the efficacy of neural transmission along the corticospinal tract [24, 25]. A reduction in ICI is important for motor performance, particularly fine movements [26]. Although there were no significant between group differences for the change in SICI in our study, the significant decrease in the vitamin D group warrants some discussion. In the rat brain, vitamin D plays a role in regulating numerous neurotransmitters, including acetylcholine, dopamine, serotonin and GABA [27, 28] and affects the expression of genes associated with GABA-ergic neurotransmission [29]. VDRs are also present on glial cells, which play a critical role in regulating the uptake and release of neurotransmitters, including GABA receptors, which are known to regulate ICI [30]. Thus, the reduction in ICI following vitamin D treatment suggests that it may modulate GABA-ergic neurotransmission within the motor cortex. This is important because GABA represents the most widely occurring inhibitory neurotransmitter within the CNS and is known to modulate muscle function [26]. Thus, it is possible that the benefits of vitamin D on muscle function may be related to neural adaptation at a cortical level by releasing corticospinal neurons from inhibition [9], although further research is needed to confirm this hypothesis.

Previous trials in older adults have shown that vitamin D treatment can improve muscle strength and function [31, 32], although these findings are not consistent [33]. This has been

attributed in part to differences in baseline 25(OH)D concentrations as well as the health and functional status of individuals. In our study, supplementation with 2000 IU/d vitamin D was associated with a modest 8-11% increase in muscle strength, but these changes did not differ significantly from the placebo group. For muscle function, we observed no effect of vitamin D on gait speed, dynamic balance or functional muscle power. These findings are consistent with some previous trials which reported that vitamin D has little or no effect on muscle strength or function in those with 25(OH)D concentrations >25-30 nmol/L [33, 34]. In our study, the mean baseline serum 25(OH)D concentration was ~47 nmol/L, and despite increasing by ~34 nmol/L after treatment, it is possible that the modest baseline levels limited our ability to detect any marked changes relative to the placebo group.

While the strengths of this study are in the double-blinded, placebo controlled design and the high participant retention and adherence to the supplements, there are limitations. First, the modest sample size may have limited our power to detect significant between group differences. Since no previous studies have investigated the effects of vitamin D treatment on neuroplasticity, it was difficult to determine the numbers needed to detect a treatment effect. However, limited data from pharmacological experiments in adults and exercise trials indicates that marked between group differences in TMS measures of neuroplasticity are evident with similar sample sizes [18, 35]. Second, we included relatively healthy older adults who displayed little evidence of impaired function; only one participant had a gait speed <1.0 m/s at baseline, which is indicative of impaired function. Third, the relatively short duration of the trial may have limited our ability to detect any significant between group differences because it can take 6-8 weeks for serum 25(OH)D levels to reach a plateau. Thus it is possible that any potential vitamin D effects on neuroplasticity may require that serum 25(OH)D levels be sustained at a higher level for a longer period of time. Finally, we did not include any

spinal cord measurements such as the H-reflex. Given the divergent monosynaptic connections of corticospinal cells onto the motoneuron pool, a measurement of spinal cord excitability would provide further information pertaining to the reduction in MEPs in our trial.

In conclusion, this study demonstrated that supplementation with 2000 IU/d vitamin D for 10-weeks has no marked effect on neuroplasticity in healthy older adults with insufficient serum 25(OH)D levels compared to placebo. However, this may be attributed to the relatively small sample size as there was a significant reduction in both corticospinal excitability and ICI and an increase in muscle strength in the vitamin D group. This suggests that vitamin D may improve the efficacy of neural transmission within the corticospinal pathway, and thereby facilitate motor output and the improvements observed in muscle strength. However, further well-designed, large-scale RCTs are needed to examine the effects on vitamin D treatment on neuroplasticity, including different doses of vitamin D, particularly in those with vitamin D deficiency. Such studies should also include brain imaging techniques (functional MRI) to examine the effects of vitamin D on brain structure and function.

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Figure 1: Study flow diagram

Figure 2: Mean ($\pm$ SE) corticospinal responses at baseline and 10 weeks for the vitamin D and placebo group. Black bar represent baseline; white bars represent 10 weeks. ^a $P < 0.05$, ^b $P < 0.01$ for within group change from baseline. All changes for intracortical inhibition are based on the log transformed data.

Figure 3: Mean ($\pm$ SE) motor evoked potential (MEP) amplitudes at baseline (solid line, black dots) and 10 weeks (dashed line, white dots) expressed as a percentage of M_{Max} in accordance to different stimulus intensity as a percentage of maximal stimulus output (MSO) in the vitamin D (top) and placebo group (bottom). ^a $P < 0.05$, ^b $P < 0.01$ within group change from baseline.

Table 1: Descriptive characteristics of the vitamin D and placebo group.

	Vitamin D	Placebo
N	13	13
Women, n (%)	8 (62%)	5 (38%)
Age (years)	66.1 ± 4.0 ^a	71.5 ± 5.7
Anthropometric		
Height (cm)	161.8 ± 8.1	167.8 ± 9.8
Weight (kg)	70.2 ± 14.4	76.9 ± 13.6
BMI (kg/m ²)	26.8 ± 5.2	27.4 ± 4.3
Dietary		
Energy (KJ/d)	7900 ± 2554	7849 ± 2306
Vitamin D (µg/d)	4.1 ± 3.7 ^a	1.6 ± 1.0
Calcium (mg/d)	842 ± 244	723 ± 372
Hormonal		
Serum calcium (mmol/L)	2.35 ± 0.05	2.36 ± 0.10
Serum 25(OH)D (nmol/L)	46.4 ± 11.4	48.5 ± 11.1
Vitamin D deficiency (<50 nmol/L), n (%)	7 (54%)	6 (46%)
Serum BDNF (ng/ml)	22.3 ± 5.0	20.9 ± 6.0
Physical Activity		
Moderate, KJ/wk	8093 ± 5598	9067 ± 4678

All values are mean ± SD or as indicated. ^a P<0.05 versus placebo

Table 2: Mean ($\pm$ SE) knee extension muscle strength and muscle function at baseline and post-intervention in the vitamin D and placebo group and the within group percentage changes and the net differences between the groups for the changes relative to baseline.

	Vitamin D			Placebo			Net difference for the change (95% CI) [#]
	Baseline	10-weeks	% Change ¹	Baseline	10-weeks	% Change ¹	
Muscle strength, N/kg							
KE 120°/s	0.97 ± 0.09	1.08 ± 0.12	3.6 ± 3.2	0.96 ± 0.06	1.12 ± 0.10	5.7 ± 2.9 ^a	-2.1 (-11.3, -0.1)
KE 180°/s	0.75 ± 0.09	0.88 ± 0.09	7.9 ± 3.1 ^a	0.74 ± 0.06	0.84 ± 0.09	4.9 ± 4.0	3.1 (-7.2, 13.4)
KE 240°/s	0.62 ± 0.08	0.77 ± 0.09	10.8 ± 3.7 ^a	0.65 ± 0.07	0.73 ± 0.07	5.7 ± 3.1	5.2 (-5.1, 15.4)
Muscle function							
Stair climb power (W)	4.48 ± 0.48	4.14 ± 0.26	2.1 ± 4.8	4.45 ± 0.39	4.44 ± 0.38	-0.2 ± 1.7	2.3 (-8.7, 13.4)
FSST (sec)	8.68 ± 0.45	8.57 ± 0.46	-10.8 ± 36	8.96 ± 0.39	8.68 ± 0.38	-29.1 ± 26	18.3 (-77.1, 113.6)
TUG (m/s)	1.72 ± 0.09	1.74 ± 0.07	2.2 ± 7.8	1.48 ± 0.15	1.51 ± 0.13	3.2 ± 12	-1.0 (-31.4, 29.4)

KE = knee extension; FSST = four square step test; TUG = timed-up-and-go test. ¹ Percentage changes in muscle strength and stair climb power are based on the absolute difference for the change relative to baseline in the log transformed data multiplied by 100. All other percentage changes are based on the absolute change in the raw data multiplied by 100. The net difference represent the difference in the unadjusted change from baseline in the vitamin D minus the placebo group. [#] All between group differences were adjusted for age and sex. ^a P<0.05 within group changes relative to baseline.

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