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Title

Effect of cholecalciferol and calcium supplementation on muscle strength and energy metabolism in vitamin D deficient Asian Indians: A randomized controlled trial
Short Title: Vitamin D supplementation and muscle strength

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Key words: Vitamin D deficiency, skeletal muscle strength

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Abstract

**Context.** Vitamin D deficiency is prevalent worldwide. Vitamin D supplementation has shown variable effect on skeletal muscle strength in the elderly with hypovitaminosis D. There is a paucity of similar data in young individuals.

**Objective.** To study the effect of cholecalciferol and calcium supplementation on muscle strength and energy metabolism in young individuals.

**Subjects.** Forty healthy volunteers (24M/16F, mean age (SD) 31.5 ± (5.0yr) with hypovitaminosis-D were randomized to either oral cholecalciferol (60,000 IU D3/week for eight weeks followed by 60,000 IU/month for four months) with 1g of elemental calcium daily or dual placebos for six months.

**Measurements:** Hand-grip and gastro-soleus dynamometry, pinch-grip strength, respiratory pressures, six-minute-walk-test and muscle-energy metabolism on $^{31}$P-magnetic-resonance-spectroscopy were assessed at baseline and after six months.

**Results.** The mean serum 25(OH)D in the supplemented and placebo groups at baseline, two and six months were 25.4 ± 9.9, 94.5 ± 53.8, 56.0 ±17.0 nmol/L and 21.1 ± 9.4, 32.8 ± 14.4, 29.7 ± 15.0 nmol/L respectively. The supplemented group gained a handgrip strength of 2.4 kg (95% C.I= 1.2-3.6); gastro-soleus strength of 3.0 Nm (95% C.I.= 0.1-5.9) and walking distance of 15.9 meters (95% C.I= 6.3-25.5) over the placebo group after adjustment for age, gender and respective baseline parameters. Muscle energy parameters were comparable at six months.

**Conclusions.** Six months of cholecalciferol and calcium supplementation results in enhanced skeletal muscle strength and physical performance despite no change in muscle energy parameters. 60,000 IU per month of cholecalciferol supplementation could not maintain 25(OH)D levels in the sufficient range.
Introduction

Vitamin D deficiency is prevalent worldwide (1,2). Up to 80-90% of urban Indians in Delhi have vitamin D deficiency with mean serum 25(OH)D levels < 25 nmol/L in winter months which relates to their skin pigmentation and lack of sunshine exposure (3). Vitamin D deficiency osteomalacia and rickets are common metabolic bone disorders in Indians (4).

Vitamin D deficiency has also been linked with several non-skeletal disorders (2). Vitamin D receptors are expressed in the skeletal muscle fibres (5). To the best of our knowledge, there have been seven randomized controlled trials assessing the effect of vitamin D with or without calcium supplementation on skeletal muscle strength (6-12). All these trials, except one, involved elderly subjects (9). Improvement in muscle strength was reported in four trials in elderly subjects who received dual supplementation of either oral cholecalciferol (6,10,11) or 1-alpha-calcidol (12) along with calcium. However, it remains unclear if the reported beneficial effects of vitamin D and calcium supplementation are limited to the elderly. The effect of such supplementation on the muscle energy metabolism has also not been assessed to date.

In this double blind randomized trial, we studied the effect of six months of combined cholecalciferol and calcium supplementation on skeletal muscle strength (hand grip and gastrosoleus), respiratory pressures, walking distance and muscle energy metabolism in healthy young individuals with low serum 25(OH)D. Combined supplementation with vitamin D and calcium was planned because of the previously reported beneficial effect of dual supplementation on muscle strength in elderly (6,10-12).

Subjects, Materials and Methods

The study group comprised of forty apparently healthy volunteers in the 20-40 years age group, including postdoctoral medical students and paramedical staff at the All India Institute of Medical Sciences, New Delhi. They were enrolled during March to May 2008 after screening a group of 65 subjects who consented for regular follow-up for six months. Pregnant women and those on any medications including vitamin D and calcium during the preceding six months were excluded. All forty subjects enrolled in the study had normal liver, renal and thyroid function tests as assessed by serum bilirubin, aspartate aminotransferase and alanine aminotransferase, blood urea, serum creatinine, serum total T4 and TSH. None of them had any complaints of muscle aches or weakness. The daily dietary intake of calories, carbohydrate, protein, fat, calcium, and phytin phosphorus (phytin-P) was assessed by a trained dietician (3,13). A validated, open-ended semi-quantitative food frequency proforma was used to collect dietary information on seven food groups.
and forty common food items consumed by Indian subjects (13,14). The information for food items consumed on daily basis was obtained by a 24-hour recall method and for items consumed less frequently by a food-frequency questionnaire. The combined information was used to calculate the average daily nutrient intake. Nutrient content of raw food was estimated using standard nutrient values of Indian foods (15). The use of semi quantitative food-frequency questionnaire for the Indian foods has been validated earlier at our centre (16).

Subjects were advised to maintain their usual physical activity and dietary pattern during the six-month study period. Direct sunlight exposure was assessed by documenting the average duration of exposure per day. The percentage of body surface area exposed was calculated by the rule of nine (17). Briefly, eleven regions of the body i.e. head and neck, each of the arms, thighs and legs, chest, abdomen, upper and lower back were assigned body surface area of 9% (totalling 99%) with 1% for the genitals (17).

Biochemical assay

At baseline, ten ml of blood was drawn under fasting conditions at 8 a.m. without venostasis and centrifuged at 4°C at 1500g. Serum samples were stored in multiple aliquots at -20°C for the estimation of serum total calcium, inorganic phosphorus and alkaline phosphatase, 25(OH)D and intact parathyroid hormone (iPTH). Blood samples were again drawn after supplementation at two months for serum 25(OH)D and at six months for all the parameters assessed at baseline. Serum 25(OH) D was measured using radio-immunoassay (Diasorin; normal range: 22.4–93.8 nmol/L) and serum iPTH was measured by electrochemiluminescence immunoassay (Roche diagnostics, Mannheim; normal range: 15–65 pg/mL). Subjects were categorized as vitamin D deficient, insufficient and sufficient based on serum 25(OH)D levels < 50 nmol/L, 50-80 nmol/L, and > 80.0 nmol/L respectively (18-20). The serum calcium, inorganic phosphorus and alkaline phosphatase levels were measured using commercial kits (Roche, Germany) and semi automated analyzer (Hitachi 4020; Boehringer, Germany) and their normal ranges were 2.0-2.6 mmol/L, 0.8-1.45 mmol/L, and 98-279 IU/L, respectively. Intra-assay and inter-assay coefficients of variation for these assays ranged between 3.5 and 5.0%.

Assessment of the skeletal muscle strength and energy at baseline and at six months

Hand-grip and gastro-soleus dynamometry, pinch grip strength, respiratory pressures, six-minute-walk test and muscle energy metabolism on 31P magnetic-resonance spectroscopy were assessed at baseline and after six months of supplementation as follows:
**Skeletal Muscle dynamometry:** Handgrip strength of the dominant limb was assessed in sitting position using a computerized dynamometer in isometric mode (JTECH Tracker system-version 4, Utah, USA). The test was performed with the shoulder in full adduction, elbow flexed at 90º, forearm in mid prone neutral position and wrist in 0 to 30º extension and the dynamometer was squeezed with maximal force three times each at two different positions. The two positions represented different resistance levels of the dynamometer. The mean of these six measurements was used to assess grip strength measured in kilograms (21). The intra-assay coefficient of variation for handgrip measurement was 3.4%.

The strength of the gastro-soleus muscle of the dominant limb was determined using a computerized dynamometer in an isokinetic mode (Biodex Multi-joint System 2 AP, NY, USA). The dynamometer was adjusted to allow foot plate movement at a speed of 180 degree/sec. The subjects were then asked to perform planter flexion on the foot plate with maximum force on three occasions in continuity and the mean of these was used as measure of the gastro-soleus strength, measured in Newton meter. The intra-assay coefficient of variation for gastro-soleus measurement was 6.7%.

**Pinch grip:** The strength of the grip between the tip of the thumb and the index finger of the dominant limb was measured using a pinch gauge instrument with the upper limb positioned as described above for hand dynamometry. The strength was recorded once and expressed in kilograms. The intra-assay coefficient of variation for pinch grip measurement was 3.9%.

**Respiratory muscle pressures:** Maximum inspiratory (MIP) and expiratory (MEP) pressures were measured using a digital respiratory pressure meter (Micro Medical Ltd, Rochester, USA). To measure the MIP, subjects were instructed to exhale to residual volume through the mouthpiece and then to inhale with maximal effort for at least two seconds. To test MEP, subjects inhaled to total lung capacity and then exhaled with maximal effort for at least two seconds. The readings displayed for MIP and MEP represented the maximum average pressure sustained over at least one second and expressed in cmH2O. The mean of three acceptable MIP and MEP readings was taken. The intra-assay coefficients of variation for MIP and MEP were 5.0%.

**Six-minute walk test along with visual analogue scale:** The subjects were asked to walk briskly for a period of six minutes along a flat and straight corridor and the distance covered was recorded in meters. At the completion of the walk, subjects were asked to grade their severity of dyspnoea on
a visual analogue scale of 0-100 mm, with the upper end of the line indicating ‘no breathlessness’ while the lower end corresponded to ‘maximum breathlessness’ (22).

**Skeletal muscle energy metabolism:**

$^{31}$P magnetic resonance spectroscopy (MRS) was performed on the calf muscles of the dominant limb for the assessment of energy metabolism using a double tuned circular $^1$H/$^{31}$P surface coil 1.5 Tesla MRI machine (Sonata, Siemens, Erlangen, Germany). The metabolites measured assessed glycolysis and mitochondrial oxidative phosphorylation energy metabolism and included phosphocreatine (PCr), inorganic phosphate (Pi), adenosine triphosphate (ATP), phosphomonoester (PME) and phosphodiester (PDE). Various spectra were acquired using a single radiofrequency pulse with 128 averages and a repetition delay of three seconds. Shimming was carried out on $^1$H nucleus and a line width of 20-25 Hz was achieved. Metabolite ratios were obtained by manual integration of the area under each peak using the software provided by the manufacturer.

**Randomization, concealment and schedule of supplementation**

Forty subjects were assigned to either the supplementation or the placebo arm using block randomization to ensure a balance of numbers recruited in the two intervention arms, with block size of four generated using STATA 9.1. Allocation concealment was maintained using sequentially numbered opaque sealed envelopes (SNOSEs). Subjects in the supplementation arm received a cholecalciferol sachet (Cadila, Pharmaceuticals Ltd, India) orally (60,000 IU D3/week for first eight weeks followed by 60,000 IU/month for four months) along with two oral tablets of calcium carbonate (each containing 500 mg elemental calcium and 250 IU D3, Elder pharmaceutical Ltd, India) given daily for six months. The corresponding placebos were lactose granules and tablets respectively; these were similar to active drugs in colour, size and packing. Subjects were advised to take a sachet of granules with a glass of milk every week and one tablet with meals, in the morning and evening. The supplementation schedule in the first two months of the study was based on our previous experience with vitamin D supplementation in healthy North Indians (23). In the current study, cholecalciferol and calcium supplementation was continued for an additional four months to provide sufficient time for the possible improvement in muscle strength.

**Compliance with supplementation**

Subjects were called every month and intake of sachets was personally supervised on all the six visits. Compliance to the intervention was carefully monitored using history and counting of
leftover tablets and sachets. Though none of the subjects returned any sachet or tablets, two of them had distributed their sachets and tablets to their children assuming the intervention to be a strength giving measure. Vitamin D status was measured at two months to assess the compliance in retrospect after the completion of the study when the randomization code was to be opened.

**Statistical analysis**

Statistical analysis was carried out using STATA 9.0 (College Station, Texas, USA). Intention-to-treat (ITT) analyses were performed and included all those who were assigned to the two intervention arms. The missing data was imputed by explicit allocation of no change in both treatment arms. Data was imputed for three of the forty subjects. These included two subjects with poor compliance and one woman who conceived during the study period. The differences in the base line parameters of vitamin D status, skeletal muscle strength and metabolite ratios between the subjects in the placebo and supplementation groups were analyzed using Student’s t test. Differences in the study parameters after six months of intervention were analyzed by analysis of covariance (ANCOVA) after adjusting for age, gender and the respective baseline parameters. All P values calculated were two tailed, and values less than 0.05 were considered significant.

**Results**

**Baseline data and change in serum 25(OH)D following intervention**

The baseline data of study subjects in the supplementation and placebo groups is shown in table 1. The average daily dietary intake of calorie, protein, calcium and sunshine exposure of all the study subjects was similar to that of healthy north Indians (3). Male to female ratio, mean age, BMI, serum total calcium, 25(OH)D, iPTH, parameters of muscle strength and ratio of various metabolites on MRS were comparable in the two groups. At baseline, all the subjects in the supplementation and placebo groups were vitamin D deficient with serum 25(OH)D levels < 50 nmol/L with mean values of 25.4 ± 9.9 and 21.1 ± 9.4 nmol/L respectively (P = 0.17).

In the supplementation group, the mean serum 25(OH)D was 94.5 ± 53.8 at two months and 56.0 ± 17.0 nmol/L at six months (P < 0.01). A similar difference was observed in the per protocol analysis (107.1 ± 47.9 and 61.7 ± 9.2 nmol/L, P < 0.01). In contrast, the mean 25(OH)D values at two months and six months were 32.8 ± 14.4 and 29.7 ± 15.0 nmol/L in the placebo group. Serum 25(OH)D values were significantly different between the supplementation and the placebo groups at two and six months (P < 0.0001 for both). There was no significant difference between serum iPTH in the supplementation and the placebo groups at six months (5.0 ± 3.1 pmol/L and 5.2 ± 1.7 pmol/L, P = 0.87).
pmol/L respectively, $P = 0.78$). Similarly, at six months there was no significant difference in serum total calcium adjusted for albumin, inorganic PO4 and alkaline phosphatase between the supplementation and the placebo groups (2.4 ± 0.2 mmol/L vs. 2.4 ± 0.1 mmol/L, $P = 0.20$; 1.1 ± 0.2 mmol/L vs. 1.1 ± 0.2 mmol/L, $P = 0.53$; 203 ± 74 IU/L vs. 180 ± 53 IU/L, $P = 0.28$ respectively).

**Muscle strength after six months of intervention**

Table 2 shows the data related to muscle strength and energy parameters as assessed by $^{31}$PMRS at six months and delta changes in these parameters in the two study groups.

Hand grip strength: At six months, the mean hand grip strength in the supplementation group was significantly higher as compared to the placebo group after adjusting for baseline differences in the age, gender and hand grip strength ($P = 0.001$). The difference in the hand-grip muscle strength at six months was 2.4 kg (95% C.I. = 1.2, 3.6) in the supplementation group over the placebo group. The delta change in the mean handgrip strength was also significantly higher in the supplementation group as compared to the placebo group.

Gastro-soleus and pinch grip strength: The mean gastro-soleus muscle strength and its delta change were significantly higher at six months in the supplementation group as compared to the placebo group. The improvement in the mean pinch strength ($P = 0.06$) and its delta change ($P = 0.07$) were comparable between the supplementation and the placebo groups.

Respiratory muscle pressures: There were no significant differences in the mean MIP and MEP and the delta change in these pressures at six months in the supplementation and the placebo groups.

Six minute walk test and dyspnoea index: The distance covered during the six minute walk test in the supplementation group at six months was significantly higher as compared to the placebo group ($P = 0.002$). The mean delta change (95% C.I) in the distance covered during six minutes walk test was significantly higher [23 (14, 32) meters] in the supplementation group as compared to the placebo group [6 (2, 10) meters, $P = 0.001$]. However, the mean dyspnoea index and its delta change were comparable in two groups at six months.

Skeletal muscle energy metabolism with $^{31}$PMRS: There was no significant difference in the mean values of PCr/Pi and PCr/ATP ratio or delta change in these parameters in the two intervention groups at six months.
The results of per protocol analyses for various parameters related to muscle strength and energy metabolism were similar to those of the intention to treat analyses.

Discussion

Several investigators have assessed the effect of vitamin D and calcium supplementation on muscle strength in the elderly with mixed results. In the present randomized double-blind placebo-controlled trial in healthy young individuals, muscle strength in both upper and lower limbs improved significantly following six months of cholecalciferol and calcium supplementation.

There have been seven randomized controlled trials assessing the effect of vitamin D with or without calcium supplementation on skeletal muscle strength in the upper and/or lower limbs (6-12). Four of these trials assessed the effect of vitamin D supplementation on hand grip strength (6-9), two of which also included daily calcium supplementation (6,7). Three of these four trials showed no significant effect of vitamin D supplementation on handgrip strength (7-9). Kenny et al observed no significant difference in the hand grip strength of 33 community dwelling elderly males following six months of oral cholecalciferol (1000 IU/day) and calcium (500 mg/day) supplementation as compared to 32 matched subjects who received calcium (500 mg/day) supplementation alone (7). Grady et al found no significant improvement in hand-grip strength following six months of calcitriol treatment (0.25 μg twice/day) in 50 elderly subjects. They explained this to be due to the higher baseline mean 25(OH) D levels (60.4 ± 35.3 nmol/L) (8). In the study by El - Hajj et al, one year of cholecalciferol supplementation in dose of 1400 IU or 14000 IU/week to two groups of young females with vitamin D deficiency failed to improve handgrip strength (9). However, Bischoff et al. showed improvement in handgrip strength by 5.5% in 33 elderly females (mean age, 85.3 years) following three months of oral cholecalciferol (800 IU/day) and calcium (1200mg/day) supplementation and an increase in mean serum 25 (OH)D level from 31 to 65 nmol/L. In contrast, the control group of 29 females on calcium supplementation (1200mg /day) alone showed no improvement in handgrip strength (6).

In the current study there was a significantly higher improvement by 7.7% in the hand grip strength after six months of supplementation as compared to the placebo unlike the three previous randomized trials; this could be because of younger age of study subjects, severity of vitamin D deficiency at baseline, the dose of vitamin D given, the duration of supplementation, the higher delta change in serum 25(OH)D and the dual nature of supplementation i.e. concomitant cholecalciferol and calcium. Serum 25(OH)D levels improved from 25.4 ± 9.9 to 56.0 ± 17.0 nmol/L at six months which was similar to that observed by Bischoff et al (6). Recently, Visser et
al and Houston et al showed that subjects with serum 25(OH)D levels in the range of 25-50 nmol/L had reduced grip strength of up to 40% as compared to those subjects with values more than 50 nmol/L (24,25).

Recently, three randomized trials have shown significant improvement in skeletal muscle strength of the lower limbs including knee extensors and hip flexors in the elderly subjects after cholecalciferol or 1-alpha-(OH)D supplementation along with calcium supplementation in comparison to control groups receiving calcium supplementation alone (10-12). The current study also showed a significantly higher improvement in the gastro-soleus muscle strength of 3.0 Newton meter following six months of supplementation as compared to the placebo group. This improved muscle strength was also reflected in the distance covered during the six minute walk test. Though the percentage gain in the total distance covered during six minutes was only 2.4% and may appear clinically negligible, it is statistically significant and, more importantly, when converted into meters, it corresponds to 15.9 metres. Ward et al showed significant positive correlation between serum 25(OH)D levels and jump velocity and jump height in 99 healthy post menarchal girls (26). Verhaar et al reported significant improvement in the distance covered during a 2 minutes walk in ten elderly women following 6 months of treatment with 1-alpha-(OH)D (0.5 μg/day) (27).

In the present study, subjects in the supplementation group covered extra distance without any significant increase in the dyspnoea score. Though respiratory pressures revealed no significant effect of cholecalciferol and calcium supplementation, we cannot exclude an improvement in diaphragmatic skeletal muscle strength as the possible cause of this improved exercise performance without any dyspnoea. Birge and Haddad showed beneficial effect of oral cholecalciferol on protein synthesis and energy metabolism in vitamin D deficient rats as indicated by increased leucine (33%) and ATP content (19%) in diaphragmatic muscle respectively (28). The measurement of transdiaphragmatic pressures by sensitive techniques might reveal changes in diaphragmatic muscle strength following cholecalciferol and calcium supplementation.

In the current study 31PMRS was carried out in view of the effect of vitamin D on phosphorus metabolism (29). However, PCr/Pi showed no significant difference at six months between the supplementation and placebo groups despite significantly increased serum 25(OH)D levels in the former. Thus, improvement in muscle strength following cholecalciferol and calcium supplementation could be due to factors other than its effect on muscle energy parameters. Muscle strength is a function of muscle mass and the contractility of the sarcomere units composed of thick (myosin) and thin (actin, tropomyosin and three troponin subunits) filaments (30). Studies in chicks have revealed increased actin and troponin C (calcium binding subunit) content of skeletal muscles following cholecalciferol supplementation (31). 1,25(OH)2D3 has been shown to increase calcium
accumulation in the sarcoplasm and calbindin D-9K content of the muscle cells of the chicks (32,33). Vitamin D supplementation induced increased intracellular calcium and troponin-C content would allow efficient contact between myosin and actin filaments and enhance skeletal muscle contraction. The role of cholecalciferol and calcium in enhancing nerve conduction and nerve-muscle junction transmission could also result in enhanced muscle contraction. We have reported reversibility of impaired nerve conduction velocity in a patient with sporadic idiopathic hypoparathyroidism following cholecalciferol and calcium supplementation (34).

While the present study provides useful information about the impact of cholecalciferol and calcium supplementation on muscle strength in young individuals, it has certain limitations. Muscle strength and energy parameters were assessed after six months of dual supplementation to provide sufficient time for the improvement in muscle strength (35). However, serum 25(OH)D levels were significantly higher at two months than at six months. It is possible that the subjects in the supplementation arm could have shown higher degree of improvement in the muscle strength and energy parameters at two months. Besides, the current study could not separate the individual effects of cholecalciferol and calcium on muscle strength. An assessment of any improvement in muscle strength with calcium supplementation alone in vitamin D sufficient population can be a subject of further studies.

The present study also provides useful information on the efficacy of 60,000 IU of cholecalciferol per month in maintaining serum 25(OH)D levels. Mean serum 25(OH)D values declined from 94.5 ± 53.8 at two months to 56.0 ± 17.0 nmol/L at six months with this dose in the supplementation arm indicating that this dose is not adequate to maintain serum 25(OH)D levels in the sufficient range.

In conclusion, six months of cholecalciferol and calcium supplementation led to significantly enhanced skeletal muscle strength in both handgrip and gastro-soleus muscles in young Indian subjects with vitamin D deficiency. This enhancement in muscle strength translated into better physical performance as seen in the significant improvement in walking distance. These finding have considerable significance in view of the wide prevalence of vitamin D deficiency in apparently healthy subjects.

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References


Table 1: Various parameters (mean and SD) at baseline in the supplementation and the placebo groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cholecalciferol and calcium (n = 20)</th>
<th>Placebo (N = 20)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (M: F)</td>
<td>13:7</td>
<td>11:9</td>
<td>0.52</td>
</tr>
<tr>
<td>Mean age (yrs)</td>
<td>32.9 ± 5.0</td>
<td>30.2 ± 4.7</td>
<td>0.08</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>25.5 ± 4.3</td>
<td>23.6 ± 3.5</td>
<td>0.13</td>
</tr>
<tr>
<td>Serum total Ca adjusted for albumin (mmol/L)</td>
<td>2.5 ± 0.2</td>
<td>2.4 ± 0.1</td>
<td>0.06</td>
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<td>Serum PO4 (mmol/L)</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.2</td>
<td>0.15</td>
</tr>
<tr>
<td>Serum alkaline phosphatase (IU/L)</td>
<td>232 ± 60</td>
<td>216 ± 70</td>
<td>0.43</td>
</tr>
<tr>
<td>Serum 25(OH)D (nmol/L)</td>
<td>25.4 ± 9.9</td>
<td>21.1 ± 9.4</td>
<td>0.17</td>
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<tr>
<td>Serum iPTH (pmol/L)</td>
<td>6.1 ± 2.5</td>
<td>7.8 ± 2.5</td>
<td>0.05</td>
</tr>
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<td>Dietary calcium intake (mg/d)</td>
<td>842 ± 366</td>
<td>910 ± 394</td>
<td>0.57</td>
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<tr>
<td>Dietary phytate:calcium</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
<td>0.47</td>
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<tr>
<td>Dietary calories (kcal/d)</td>
<td>1946 ± 403</td>
<td>2095 ± 631</td>
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<tr>
<td>Dietary protein (gm/d)</td>
<td>58 ± 16</td>
<td>68 ± 24</td>
<td>0.14</td>
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<tr>
<td>Sun exposure time (minutes/d)</td>
<td>28 ± 16</td>
<td>26 ± 12</td>
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<tr>
<td>Sun exposure, body surface area (%)</td>
<td>17 ± 3</td>
<td>16 ± 4</td>
<td>0.39</td>
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<tr>
<td><strong>Muscle strength related parameters</strong></td>
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<tr>
<td>Handgrip strength (kg)</td>
<td>31.0 ± 9.7</td>
<td>29.7 ± 7.1</td>
<td>0.65</td>
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<tr>
<td>Pinch strength (kg)</td>
<td>6.3 ± 1.5</td>
<td>5.9 ± 1.3</td>
<td>0.36</td>
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<td>Gastro-soleus strength (Newton meter)</td>
<td>18.0 ± 8.1</td>
<td>17.7 ± 7.6</td>
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<td>Maximum Inspiratory Pressure (cm/H2O)</td>
<td>83.9 ± 24.1</td>
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<td>Maximum Expiratory Pressure (cm/H2O)</td>
<td>98.8 ± 21.9</td>
<td>89.1 ± 25.3</td>
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<td>6 min walk test (distance in meters)</td>
<td>650 ± 82</td>
<td>665 ± 93</td>
<td>0.58</td>
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<td>Visual analogue scale (dyspnoea %)</td>
<td>31.5 ± 10.9</td>
<td>33.5 ± 11.8</td>
<td>0.58</td>
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<td><strong>31PMRS muscle energy related parameter</strong></td>
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<tr>
<td>PCr/Pi</td>
<td>9.83 ± 2.97</td>
<td>9.82 ± 3.30</td>
<td>0.99</td>
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Table 2: Comparison of parameters in the supplementation and the placebo groups at six months

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cholecalciferol and calcium (n = 20)</th>
<th>Placebo (n = 20)</th>
<th>Difference in the means (95% C.I.)</th>
<th>P-value</th>
<th>Difference in the means (95% C.I.) *</th>
<th>P-value*</th>
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<td>Unadjusted</td>
<td>Adjusted</td>
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<tr>
<td>Muscle strength related parameters</td>
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<td>Handgrip strength (kg)</td>
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<tr>
<td>At six month</td>
<td>34.0 ± 10.3</td>
<td>30.0 ± 7.5</td>
<td>4.0 (-1.8, 9.7)</td>
<td>0.17</td>
<td>2.4 (1.2, 3.6)</td>
<td>0.001</td>
</tr>
<tr>
<td>Delta change (95% C.I.)</td>
<td>3.1 (2.0, 4.1)</td>
<td>0.3 (-0.4, 1.0)</td>
<td>2.8 (1.5, 3.8)</td>
<td>0.001</td>
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<tr>
<td>Pinch strength (kg)</td>
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<tr>
<td>At six month</td>
<td>6.5 ± 1.5</td>
<td>5.9 ± 1.4</td>
<td>0.6 (-0.4, 1.5)</td>
<td>0.22</td>
<td>0.2 (-0.01, 0.4)</td>
<td>0.06</td>
</tr>
<tr>
<td>Delta change (95% C.I.)</td>
<td>0.2 (0.04, 0.3)</td>
<td>0.02 (-0.1, 0.1)</td>
<td>0.2 (-0.01, 0.4)</td>
<td>0.07</td>
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<tr>
<td>Gastro-soleus strength (Newton meter)</td>
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<tr>
<td>At six month</td>
<td>23.5 ± 12.2</td>
<td>19.8 ± 9.7</td>
<td>3.7 (-3.3, 10.8)</td>
<td>0.29</td>
<td>3.0 (0.1, 5.9)</td>
<td>0.04</td>
</tr>
<tr>
<td>Delta change (95% C.I.)</td>
<td>5.5 (2.7, 8.3)</td>
<td>2.1 (0.4, 3.8)</td>
<td>3.4 (0.3, 6.6)</td>
<td>0.04</td>
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<tr>
<td>MIP (cm/H$_2$O)</td>
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<tr>
<td>At six month</td>
<td>88.3 ± 24.2</td>
<td>80.6 ± 23.8</td>
<td>7.7 (-7.7, 23.0)</td>
<td>0.32</td>
<td>0.04 (-3.4, 4.2)</td>
<td>0.84</td>
</tr>
<tr>
<td>Delta change (95% C.I.)</td>
<td>4.3 (1.3, 7.4)</td>
<td>3.0 (0.6, 5.4)</td>
<td>1.4 (-2.4, 5.1)</td>
<td>0.47</td>
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<tr>
<td>MEP (cm/H$_2$O)</td>
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<tr>
<td>At six month</td>
<td>104.8 ± 25.5</td>
<td>91.2 ± 27.5</td>
<td>13.5 (-3.4, 30.5)</td>
<td>0.11</td>
<td>3.8 (-1.1, 8.7)</td>
<td>0.13</td>
</tr>
<tr>
<td>Delta change (95% C.I.)</td>
<td>5.95 (1.02, 10.9)</td>
<td>2.1 (0.3, 3.8)</td>
<td>3.9 (-1.3, 9.0)</td>
<td>0.14</td>
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<tr>
<td>6 min walk test (distance in meters)</td>
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<tr>
<td>At six month</td>
<td>673 ± 93</td>
<td>671 ± 96</td>
<td>1.7 (-58.8, 62.3)</td>
<td>0.95</td>
<td>15.9 (6.3, 25.5)</td>
<td>0.002</td>
</tr>
<tr>
<td>Delta change (95% C.I.)</td>
<td>23 (14, 32)</td>
<td>6 (2, 10)</td>
<td>17.0 (7.3, 26.7)</td>
<td>0.001</td>
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<tr>
<td>Visual analogue scale (dyspnoea %)</td>
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<tr>
<td>At six month</td>
<td>29.5 ± 9.4</td>
<td>32.5 ± 11.2</td>
<td>-3.0 (-9.6, 3.6)</td>
<td>0.36</td>
<td>-2.6 (-7.2, 1.9)</td>
<td>0.25</td>
</tr>
<tr>
<td>Delta change (95% C.I.)</td>
<td>2 (-5.6, 1.6)</td>
<td>1 (-4.4, 2.4)</td>
<td>1.0 (-5.8, 3.7)</td>
<td>0.67</td>
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<tr>
<td>31PMRS muscle energy related parameter</td>
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<tr>
<td>PCr/Pi at six month</td>
<td>8.94 ± 2.26</td>
<td>9.73 ± 2.97</td>
<td>-0.78 (-2.47, 0.90)</td>
<td>0.35</td>
<td>-0.78 (-2.45, 0.90)</td>
<td>0.35</td>
</tr>
<tr>
<td>Delta change (95% C.I.)</td>
<td>-0.9 (-2.24, 0.46)</td>
<td>-0.09 (-1.70, 1.50)</td>
<td>0.63 (-2.64, 1.37)</td>
<td>0.53</td>
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</tr>
</tbody>
</table>

Data is shown as mean ± S.D. MIP: mean inspiratory pressure; MEP: mean expiratory pressure.
Delta change represents average difference from baseline to six months.
* Difference in means after adjusting for age, gender and respective parameter at baseline, between the supplemented and the placebo groups.