Effect of cholecalciferol supplementation on muscle strength in healthy volunteers with low serum 25(OH)D: A double blind randomized placebo controlled trial

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Objective: To investigate the effects of six months of cholecalciferol and calcium supplementation on the skeletal muscle strength and muscle energy metabolism using ^{31}P magnetic resonance spectroscopy (^{31}PMRS) in healthy volunteers with low serum 25-hydroxy vitamin D [25(OH)D].

Introduction: Vitamin D deficiency (VDD) is common in several parts of the world [1]. Up to 80-90% of urban Indians in Delhi have VDD with mean serum 25(OH)D levels as low as <25 nmol/L in winter [2]. Besides metabolic bone disorders, VDD is also associated with increased prevalence of malignancies and autoimmune disorders such as type-1 diabetes and multiple sclerosis. Recently, the association between 25(OH)D deficiency and thyroid autoimmunity has been demonstrated [3]. Vitamin D receptors are also expressed in the skeletal muscle and experimental studies have demonstrated increased accumulation of leucine, ATP and inorganic phosphorus into diaphragm muscle following vitamin D treatment [4]. In open labeled studies, vitamin D supplementation resulted in improved muscle strength of patients with osteomalacia and elderly subjects with low serum 25(OH)D levels [5]. The effect of VDD on the muscle energy metabolism using ^{31}PMRS has not been assessed to date in VDD. In the present double blind randomized placebo controlled trial (NCT00682214), we report the effects of six months of cholecalciferol and calcium supplementation on the skeletal muscle strength and muscle energy metabolism using ^{31}P magnetic resonance spectroscopy (^{31}PMRS) in healthy volunteers with low serum 25(OH)D.

Subjects, Materials and Methods: Forty apparently healthy volunteers (age - 20-40 years) were block randomized to active or placebo arm in a double blind manner. The active arm consisted of cholecalciferol sachet given orally (60,000 IU/week for initial eight weeks followed by 60,000 IU/month for four months) along with the two tablets of calcium carbonate (each containing 500 mg elemental calcium and 250 IU D_3) given daily for six months. At baseline, serum total calcium, inorganic phosphorus and alkaline phosphatase, 25(OH)D and intact parathyroid hormone (iPTH) were estimated. Also hand grip strength, gastrosoleus muscle strength , maximum inspiratory (MIP) and expiratory pressures (MEP) and distance covered by each subject during six minutes walk test and degree of breathlessness felt at the end of the walk as quantified by visual analogue scale (VAS) were recorded. ^{31}PMRS was performed in calf muscle of the dominant limb at 1.5 Tesla MRI scanner (SONATA, Siemens) using a double tuned circular ^{1}H/^{31}P surface coil. Spectra were acquired using a single radiofrequency pulse with 128 averages and a repetition delay of three seconds. Metabolite ratios were obtained by manual integration of the area under each peak using the software provided by the manufacturer. Reassessment of skeletal muscle strength and ^{31}PMRS studies was performed at completion of six months of supplementation. Statistical Analysis was carried out using STATA 9.0 (College Station, Texas, USA). Intention-to-treat (ITT) analyses were performed as efficacy analyses for primary and secondary outcomes. For primary outcome, per protocol (PP) analysis was also performed. The differences in base line parameters related to vitamin D status, skeletal muscle strength and metabolite ratios between subjects in the placebo and active treatment arms were analyzed using Student's *t* test. Differences between primary and secondary outcome after six months of intervention were analyzed by analysis of covariance (ANCOVA) after adjusting for age, serum calcium, and serum iPTH since these were different in the two groups at baseline. All *P* values calculated were two tailed, and value less than 0.05 was considered significant. Institute ethics committee approved the study.

Results: At baseline, all the subjects in the cholecalciferol and placebo intervention groups were vitamin D deficient with serum 25(OH)D levels with mean values of 25.4 ± 9.9 and 21.1 ± 9.4 nmol/L respectively ($P = 0.17$) that increased significantly to 61.7 ± 9.2 nmol/L in the cholecalciferol group following intervention, at six months, while in the placebo group, the mean serum 25(OH)D values was 29.7 ± 15.0 nmol/L. At six months, the mean hand grip strength in cholecalciferol supplemented group was significantly higher as compared to placebo group in both ITT and PP analysis ($P = 0.003$ and 0.0001 respectively). The mean gastrosoleus muscle strength, distance covered during six minute walk were significantly higher at six months in the cholecalciferol group as compared to placebo group. There were no significant differences in the mean MIP and MEP values and the mean values of PCr/Pi and PCr/ATP ratio in the two groups at six months (Table 1).

Table 1: Effect of six months of cholecalciferol supplementation on handgrip strength (primary outcome) as compared to the placebo group.

Parameters	Cholecalceferol (n=20)	Placebo (n=20)	Difference between means (95% C.I.)	*P*-value
Handgrip strength (kg) (Mean ± S.D.)	36.5±8.7	30.0±7.5	6.5 (1.1, 11.9)	0.02
ANCOVA analysis			3.3 (1.8,4.9)	0.001
Gastrosoleus strength (Newton meter)	23.5±12.2	19.8±9.7	4.3 (1.0, 7.6)	0.01
MIP (cm/H_2O)	88.3±24.2	80.6±23.8	-0.7 (-5.0,3.7)	0.76
MEP (cm/H_2O)	104.8±25.5	91.2±27.5	3.3 (-2.7,9.3)	0.27
6 min walk test (distance in meters)	673±93	671±96	19.1 (8.5, 29.7)	0.001
Visual Analogue scale (dysponea %)	29.5±9.4	32.5±11.2	-3.0 (-8.1, 2.0)	0.22
PCr/Pi ratio	8.94±2.26	9.73±2.97	0.07 (-1.71, 1.86)	0.09

Discussion: In the present randomized double blind placebo controlled trial, handgrip strength improved significantly following six months of cholecalciferol supplementation in ITT as well as in PP analysis. Bischoff *et al.* have also shown improvement in handgrip strength of elderly females by 5.5% following three months of cholecalciferol supplementation and associated increase in serum 25 (OH)D levels [6]. Our study also showed a significant improvement in gastrosoleus muscle strength following cholecalciferol supplementation which was also reflected in the distance covered during six minute walk test. Ward *et al* also documented significant positive correlation between 25(OH)D levels with jump velocity, jump height and muscle power of lower limb in apparently healthy post menarchal girls [7]. Experimental studies have revealed increased actin and troponin C (calcium binding subunit) content of the skeletal muscle following vitamin D supplementation [8]. Besides, vitamin D can enhance ATP dependent accumulation of calcium in the sarcoplasm and Calbindin D-9K content of the muscle cell [9]. In the present study, PCr/Pi showed no significant differences between the cholecalciferol supplemented and placebo groups. Thus, improvement in muscle strength following cholecalciferol could be due to factors other than its effect on change in muscle energy and phosphorus parameters.

References:

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