Combination of Alfacalcidol with Calcium Can Improve Quadriceps Muscle Strength in Elderly Ambulatory Thai Women Who Have Hypovitaminosis D: A Randomized Controlled Trial

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Objective: *The purpose of this study was to evaluate the efficacy of alfacalcidol and calcium on the improvement of muscle strength in ambulatory elderly Thai women in age group of 65 or more who have hypovitaminosis D.*

Material and Method: *Seventy-two postmenopausal women age 65 years or more were enrolled to this study. Blood was collected from all participants for measured of 25(OH)D_3, intact PTH and vitamin D receptor (VDR) genotypes. After blood collection, the quadriceps muscle strength was measured using the isokinetic dynamometer device. There were 42 subjects who satisfy the eligible criteria and agreed to participate in the experimental randomized controlled study. These subjects were randomized into two groups, one received calcium 1500 g/day combined with alfacalcidol 0.5 mg/day. Another group received calcium 1500 g/day with placebo.*

Results: *After 12 weeks of intervention, 40 subjects had the second muscle strength measurement (2 dropped out). By ANCOVA analysis, there were significant improvement of muscle strength in the group that received alfacalcidol compared to placebo in both 30°/sec (20.28 vs.16.29, p = 0.025) and 60°/sec (20.32 vs. 15.05, p = 0.002) angular velocities.*

Conclusion: *Daily doses of 0.5 mg alfacalcidol with calcium effectively improved muscle strength in elderly Thai women who had low level of 25(OH)D_3 compared to calcium alone.*

Keywords: *Vitamin D metabolite, Hypovitaminosis D, Alfacalcidol, Muscle strength, Falls*

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The pivotal roles of calcium and vitamin D in physiology of many systems in our body are generally known. Vitamin D_3 or cholecalciferol is synthesized from 7-dehydrocholesterol in the human skin in response to sunlight. The active hormone, 1, 25-dihydroxyvitamin D_3 (1, 25$(OH)_2D_3$), is produced by sequential hydroxylations of vitamin D_3 in the liver (25-hydroxylation) and the kidney (1a-hydroxylation). 1, 25$(OH)_2D_3$ acting through the vitamin D receptor (VDR), by a genomic mechanism which is identical to the classical steroid hormones, regulate target gene transcription. The traditional actions of 1, 25$(OH)_2D_3$ are to enhance calcium and phosphate absorption from the intestine in order to maintain normal concentration of these essential ions in serum and provide normal mineralization. Calcium and vitamin D deficiency is the most important risk factor in the pathogenesis of osteoporosis. As well, vitamin D deficiency also lead to secondary hyperparathyroidism, increase bone

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turnover and increase bone loss. Apart from the well-known effects on bone metabolism, this condition is also associated with the decrease in muscle strength. The increase bone of fragility and increased number of falls due to impaired muscle function are known as risk factors of hip fractures[1]. Postural instability has also been identified as a risk factor for Colles' fracture[2]. The percentage of elderly people who fall down and hurt themselves increases steeply in those older than 70 years, and over 90% of hip fractures in elderly people occur as a result of a fall. Impaired balance and increased body sway are important causes of fall[3].

Elderly women are prone to develop vitamin D deficiency because of various risk factors: decreased dietary intake, diminished sunlight exposure, reduced skin thickness, impaired intestinal absorption, and impaired hydroxylation in liver and kidneys. As mention above, 1, $25(OH)_2D_3$ or active vitamin D metabolites acting on classical target organs through the genomic pathway via its nuclear receptor VDR is considered to be a hormone rather than a vitamin[4]. The serum concentration of 25 hydroxyvitamin D_3 ($25(OH)D_3$), the circulating form of vitamin D metabolites, is 1,000 times that of serum 1, $25(OH)_2D_3$, and this excess concentration constitutes a storage facility similar to that of other steroid hormones[4]. Although it is generally agreed that vitamin D status is most accurately reflected by serum $25(OH)D_3$ level, the evidence regarding adequate serum concentration is inconclusive.

However, myopathy has been shown to be a prominent and common symptom of vitamin D deficiency. Some new data indicate that severely impair muscle functions may present even before biochemical signs of bone disease develops[5]. 1, $25(OH)_2D_3$ affects muscle cell metabolism through various pathways. The genomic pathway through the VDR (vitamin D receptor), in muscle fibers which results in changes in gene transcription and subsequences protein synthesis, is found to influence many functions of muscle cell such as calcium uptake, phosphate transport across the membrane, phospholipid metabolism and to mediate cell proliferation and subsequently differentiation[6-9]. The importance of the VDR gene polymorphisms in the development of osteoporosis has continuously increases interest. The correlation between VDR gene polymorphisms and muscle strength was also found. Geusens et al[10], with the use of specific restriction endonuclease, find that VDR gene polymorphisms have some correlation to muscle function. In non-obese, elderly women, a 23% difference in quadriceps strength and 7% difference in grip strength between the 2 homozygote types of a restriction site are found[10].

The evidence indicates that 1, $25(OH)_2D_3$ possibly acts on membrane receptor, the non-genomic pathway, which rapidly cause further interacting with secondary messengers and subsequently activates muscle cell, resulting in enhance calcium uptake through the calcium channels[11,12]. Although the possible interaction between vitamin D status and muscle strength has been proposed for decades, only few human studies and clinical trials have been performed in this field.

Chapuy et al[13] inspired many investigators to conduct the studies on the muscular effect of vitamin D. In the group of 3,270 elderly nursing home residents, supplementation with vitamin D (800 IU/d) and calcium (1200 mg/d) led to a 43% reduction in hip fracture risk after 18 months of treatment[13]. The bone density at the proximal femur increased by 2.7% in the vitamin D-calcium group and decreased by 4.6% in the placebo group[13]. This reduction in hip fracture incidence was somewhat surprising as the change in BMD was modest. The re-evaluation after 3 years continued to show a 25% reduction in the incidence of hip fracture. In addition, a study performed in men and women at the age of or more 65 living in community, dietary supplementation with vitamin D and calcium increased BMD moderately while significant reduction in non-vertebral fractures was observed[13]. Twenty-six subjects in the placebo group compared with 11 subjects in the calcium and vitamin D group had non-vertebral fractures ($p = 0.02$)[14]. It seems unlikely that the anti-fracture efficacy of vitamin D and calcium is attributable to their effect on BMD alone because since the reduction of fracture risk tends to forward the improvement of bone mass induced by calcium and vitamin D. The action of vitamin D and calcium on decreased secondary hyperparathyroidism and decreased bone turnover were also considered, but another interesting explanation was the efficacy in reduction of falls by improved muscle function.

Clinical studies of correlation between vitamin D status and muscle function were well demonstrated. One study of an elderly population (aged 65-95) of whom 12% of women and 18% of men had a serum $25(OH)D_3$ concentration < 30 nmol/L (12 ng/ml), a significant correlation was found between vitamin D metabolites and leg extension power[15]. This finding agreed with the study by Mowe et al[16], in which the association between serum vitamin D

metabolites, $25(OH)D_3$, and muscle function was examined. In this study, serum $25(OH)D_3$ concentrations were significantly lower in those subjects who had less handgrip strength was unable to climb stairs, did no outdoor activity, and fell in the previous month[16]. On the other hand, Boonen et al[17] investigated the correlation between muscle function and serum levels of 1, $25(OH)_2D_3$ in 245 elderly women (70-90 years). No correlation could be demonstrated. $25(OH)D_3$ levels were not reported in this study.

Several studies show the clinical improvement in muscle strength and function accompanying with vitamin D therapy, whereas some studies do not. In one treatment studied by Grady et al[18], it failed to demonstrate any muscular effects of treatment with 0.5 mg calcitriol daily in 98 male and female volunteers who were over 69 years old. However, serum levels of $25(OH)D_3$ among these participants were above 60 nmol/L (24 ng/ml). Verhaar et al[19] their study performed a contrary result. They studied in 10 vitamin D deficient (serum $25(OH)D_3 < 20$ nmol/L or 8 ng/ml), elderly women (mean age 76) who were treated with 0.5 mg/d of alfacalcidol. Both knee extension strength and walking distance improved significantly in those women. Bischoff et al[20] demonstrated a reduction in falls after treatment with vitamin D and calcium in elderly institutionalized women. The women with a mean age of 85 received either 1,200 mg of elemental calcium or 1,200 mg of elemental calcium plus 800 IU of vitamin D. The number of falls before the treatment that subtracted from the number of falls during the treatment period was significantly lower in the calcium and vitamin D group ($p < 0.01$)[20]. Glerup et al[5] studied the treatment effect of vitamin D in veiled Arab women living in Denmark (n = 55) ($25(OH)D_3 = 6.7 \pm 0.6$ nmol/L). They demonstrated a 34% reduction in muscle power determined by voluntary knee extension (MVC) when compared with the control (n = 22) group taking normal vitamin D levels. A series of ergocalciferol injections (100,000 IU weekly for one month follow by 100,000 IU monthly for six months) and daily calcium supplement of 1,200 mg were given. MVC increased by 13% after three months and by 24% after six months ($p < 0.02$)[5]. Further, MVC correlated significantly with serum levels of $25(OH)D_3$ ($r = 0.34$, $p < 0.01$) and PTH ($r = -0.33$, $p < 0.001$), but not with 1, $25(OH)_2D_3$. In multivariate regression analysis, only $25(OH)D_3$ was found to be significant[5].

Very few studies in effect of hypovitaminosis D have been conducted in Thailand. Chailurkit et al[21] reported in 158 normal Thai volunteers, 81 women and 77men between 20 and 80 years old. They found that mean level of serum $25(OH)D_3$ was 42.2 ng/ml (106 nmol/L), and they concluded that no evidence of vitamin D deficiency in ambulatory Thais. But only 39 cases of postmenopausal women were enrolled in this study. Another study by Soontrapa et al[22] in the elderly Thai women living in Khon Kaen province (mean age of 69 years old) found a significantly inverse relationship between serum $25(OH)D_3$ and PTH concentration. Serum PTH concentration started to increase steeply when serum $25(OH)D_3$ concentration declined < 30 ng/ml. As a result, the prevalence of hypovitaminosis D in this population would be at least 34.9%[22]. The authors discussed that the belief that there are no hypovitaminosis D or vitamin D deficiency in tropical or subtropical countries should be reconsidered[22]. This result was corresponded to some studies in other tropical Asian area[23,24]. If hypovitaminosis D has a significant prevalence in Asian people and causes a significant effect on muscle strength, it can increase additional risk to fall and subsequence fractures. The study to evaluate the correlation between serum $25(OH)D_3$ concentration and muscle strength in these elderly women population as well as a solid study to evaluate the response of them to the treatment with vitamin D metabolite in terms of muscle strength have not been established.

Material and Method

This study was designed as a randomized double-blinded placebo-controlled experimental trial to answer the primary research question: can orally given alfacalcidol effectively improve quadriceps muscle strength in ambulatory elderly Thai women at the age of 65 or more, who have hypovitaminosis D? The concept of "hypovitaminosis D" or vitamin D insufficiency needs to be distinguished from "vitamin D deficiency" which is a very low serum $25(OH)D_3$ and leads to osteomalacia and severely impaired muscle function. We defined hypovitaminosis D as a level of serum $25(OH)D_3$ that influenced calcium homeostasis and compensatory increase in PTH level. According to Soontrapa et al[21] studying in Thai population, a level of serum $25(OH)D_3$ that equal to or lower than 30 ng/ml (75 nmol/L) was the definition.

Subjects enrollment and blood testing

From January 2004 to December 2004, Thai postmenopausal 65-year old or more women age who came to Osteoporosis Clinic at Department of

Orthopedics, Phramongkutklao Army Hospital were screened for this study. All of them were able to walk alone and none of them engaged in regular physical exercise programs. These ambulatory elderly women age that came to attend, were selected according to the inclusion and exclusion criteria. We excluded those who had the evidence of chronic medical diseases such as diabetes mellitus or other endocrine disorders requiring therapy, systemic lupus erythematosus, rheumatoid arthritis or other severe arthritic diseases, congestive heart failure, coronary artery disease, angina, or myocardial infarction, neurogenic diseases: stroke, Parkinsonism or dementia. We also excluded those who had the evidence of liver or renal impairment, who had history of cancer within 5 years, who had the visual abnormality which required major treatment or could not corrected by eyeglasses and those who had prior use of fluoride, androgen, estrogen, calcitonin, or corticosteroids within 3 months, bisphosphonates within 6 months and prior use of investigation drug (vitamin D metabolites and calcium supplement) within 1 month. There were 72 women who passed all exclusion criteria and were consented to the study. All of them were sent for blood testing including serum $25(OH)D_3$, serum PTH and VDR genotyping. Venous blood was collected from all participants for analysis of serum $25(OH)D_3$, serum intact PTH and vitamin D receptor (VDR) genotypes. Serum $25(OH)D_3$ was analyzed by Radioimmunoassay (RIA) method (DiaSorin, MN, USA) and serum intact parathyroid hormone was analyzed by Electrochemiluminescence (ECL) technique (Roche diagnostic, Switzerland). Genotyping of VDR was analyzed by specific restriction endonuclease and PCR amplification technique. VDR genotypes were named as follows: BB (absence of the *Bsm*-I restriction site on both alleles), Bb (heterozygous for the restriction site), bb (presence of the restriction site on both alleles).

Measurement of muscle strength

After blood testing, all participants were sent to measure the quadriceps muscle strength using the isokinetic dynamometer device (Biodex, USA) (Fig. 1). The measurement was performed by a single physical therapist that blinded to treatment status of the subjects. Isokinetic dynamometer is an electromechanical device with a lever arm contains a transducer that measures force as torque when the subject pushes or pulls on the lever arm and a goniometer that measures joint angle. We measured muscle strength of the quadriceps muscle on both sides at specific angular velocity by choosing the 30°/sec and 60°/sec as they represented the velocity in slow walking and normal walking of elderly subjects, respectively. Isokinetic peak torque in extension of the quadriceps muscle (muscle strength) expresses in Newton-meters (N-m). All measurements were done in both side (left and right) in each angle of velocity and we used their average as a result of each subject, each angle. All values were managed as continuous numerical variables.

Allocation of treatment and sample size

After blood testing and quadriceps muscle measurement, only the elderly women who had serum

Fig. 1 Isokinetic Dynamometer Device (Biodex, USA) for muscle strength measurement

$25(OH)D_3 \leq 30$ ng/ml were enrolled to the experimental randomized controlled trial (RCT). All the allocation was concealed with a sealed, opaque envelope kept by the assistant nurse at the clinic. Those women were allocated to either the treatment group who received alfacalcidol 0.5 mg/d (Bon-one, Teijin Co Ltd, Japan) and calcium carbonate 1,500 mg/d or the control group, who received placebo having the same characteristic as true active drugs (Teijin Co Ltd, Japan) and calcium carbonate 1,500 mg/d. The number of subjects in each group was 20 subjects. There had been no prior study about the normal value of isokinetic peak torque (PT) of knee extensor muscle in Thai population, thus we calculated from the study by Aquino et al[25] which the mean isokinetic peak torque (PT) of knee extensor was 76.97 ± 14.59 N-m. We expect the difference between the alfacalcidol group and the controlled one (m_1-m_2) about 20%, and using an error of 0.05 (two-tailed) and b error of 0.2 (power 80%).

Outcome measurement and statistical analysis

In each subject, the measurement of all muscle strength variables were done in 2 times, before beginning the intervention as the baseline data (together with blood testing), and at 12th week just before the intervention stopped. All these muscle strength variables were expressed as the mean muscle strength at 30°/sec (N-m) and at 60°/sec (N-m). We used Analysis of Covariance (ANCOVA) to adjust the baseline muscle strength as a covariate. The primary outcome of interest was to compare the muscle strength in treatment with alfacalcidol group and the placebo groups at 12th week.

Ethical consideration

Alfacalcidol is registered by Thai FDA as the drug for improvement of calcium absorption and treatment of osteoporosis. From many studies, this medication can be used with very few adverse effects. The hypercalcemia after using of this drug is rarely reported because this drug should have metabolites at liver before becoming an active form.

The protocol must be reviewed and approved by the Royal Thai Army Medical Department Institutional Review Committee. All eligible subjects will be informed the details of the study by research assistants, who have to explain the protocol thoroughly about the objective, method of study, treatment outcomes, potential adverse events and also the subjects' right to refuse to participate or withdraw from this study at any time without affecting their proper medical care.

A signed informed consent was obtained from the subjects without enforcement. There was some ethical consideration for the control group, which received placebo. Normally, the subjects should have no significant symptom at all. The trial period was actually short and all of the subjects were received calcium carbonate in dose of 1,500 mg/d along this period.

Results

There were 72 postmenopausal women aged 65 years or more involved in this blood screening and muscle strength measurement. Table 1 shows the baseline and demographic data of all participants in this study. Participants' age varied from 65 to 84 years old with the mean age of 70.6. The average body weight

Table 1. Baseline and demographic data in all participants (72 cases)

	Mean	SD	Min-Max
Age (yr)	70.60	4.30	65.00-84.00
Weight (kg)	60.80	6.70	45.80-71.00
Serum $25(OH)D_3$ (ng/ml)	27.99	7.67	12.00-70.83
Serum iPTH (pg/ml)	45.14	19.23	15.04-94.50
VDR genotype [n (%)]			
BB	4 (5.6%)		
Bb	18 (25.0%)		
bb	50 (69.4%)		
Mean baseline muscle strength at 30°/sec (N-m)	18.95	7.36	6.40-44.00
Mean baseline muscle strength at 60°/sec (N-m)	17.44	8.55	4.70-55.15

Serum iPTH = serum intact parathyroid hormone, VDR = vitamin D receptor

was 60.8 kilograms. The mean serum $25(OH)D_3$ was 27.99 ng/ml and the mean serum intact PTH was 45.14 pg/ml. The mean baseline quadriceps muscle strength measured by isokinetic dynamometer at 30°/sec was 18.95 ± 7.36 N-m, and at 60°/sec was 17.44 ± 8.55 N-m. The frequency distribution of VDR genotype in this study was BB 5.6%, Bb 25% and bb 69.4%. If we categorized these baseline characteristics according to the serum level of $25(OH)D_3$ into a group of serum $25(OH)D_3 > 30$ ng/ml and a group of serum $25(OH)D_3 \leq 30$ ng/ml, the distribution of these baseline data was shown as Table 2. According to the study by Soontrapa et al[22], vitamin D insufficiency or hypovitaminosis D was defined as the population which had serum $25(OH)D_3$ equaling or lower than 30 ng/ml. In this study the percentage of hypovitaminosis D was very high ; it was 63.9% (46 in 72 cases). The mean age, weight, serum iPTH and initial muscle strength at both angular velocities were not different between two groups. There was slightly more frequency of bb genotype in the group of serum $25(OH)D_3$ 30 ng/ml.

There were 46 elderly subjects who had serum $25(OH)D3 \leq 30$ ng/ml, and 42 of them who were willing to participate into this study. These women were randomly allocated into either treatment (alfacalcidol 0.5 μg/d + calcium carbonate 1,500 mg/d) or control (placebo + calcium carbonate 1,500 mg/d) groups by simple randomization. There had been two subjects withdrawn from the study before it was finished. One subject discontinued the intervention drug at the early stage (< 4 weeks); the other sustained an ankle fracture. These the number of subjects who received the second muscle strength measurement at 12th week was 40. After opening the concealment, we found that the two dropped out subjects were in the placebo group; the number of subjects in the placebo group was 19 while the number of subjects in the treatment group was 21.

Randomized, controlled study results

Table 3 shows the baseline and the post-intervention results of this study. The mean age, weight, serum $25(OH)D_3$, serum iPTH and initial muscle strength were not significant different. The VDR genotype in the placebo group had more frequency in bb population than in the treatment group. After 12th week of intervention, there was an improvement of muscle strength in the treatment group in both angular velocities compared to the placebo group which the muscle strength decreased in both angular velocities. We used Analysis of Co-variance (ANCOVA) to compare the results of muscle strength at 12th week between the alfacalcidol and the placebo group. In this model, we set the baseline muscle strength as a covariate. The results from the ANCOVA show the statistically significant improvement of muscle strength ($p = 0.025$ in 30°/sec and $p = 0.002$ in 60°/sec) in the alfacalcidol group compare to the placebo one in both angular velocity (Table 4, 5). Fig. 2-3 show the box-plot distribution of muscle strength at 12th week and muscle strength at baseline showed the improvement in muscle strength in both angular velocity.

The results regarding adverse events and complications were summarized in Table 6. No serious adverse events were reported. All subjects except two of them participated in this study till its finish. The

Table 2. Baseline and demographic data expressed as the mean ± SD in all participants (72 cases) categorized by level of serum $25(OH)D_3$ into the group of > 30 ng/ml and the group of ≤ 30 ng/ml

	$25(OH)D_3 > 30$ ng/ml	$25(OH)D_3 \leq 30$ ng/ml
Number	26	46
Age (yr)	69.80 ± 4.20	71.00 ± 4.30
Weight (kg)	60.93 ± 5.65	60.78 ± 7.36
Serum iPTH (pg/ml)	47.64 ± 19.62	43.73 ± 19.08
VDR genotype [n (%)]		
BB	2 (7.7%)	2 (4.3%)
Bb	8 (30.8%)	10 (21.7%)
bb	16 (61.5%)	34 (73.9%)
Mean baseline muscle strength at 30°/sec (N-m)	18.73 ± 7.25	19.07 ± 7.50
Mean baseline muscle strength at 60°/sec (N-m)	17.63 ± 9.54	17.33 ± 8.04

Serum iPTH = serum intact parathyroid hormone, VDR = vitamin D receptor

Table 3. Baseline, demographic data expressed as mean ± SD, and result of improvement in muscle strength* in particpants group ≤ 30 ng/ml who enrolled in experimental RCT

	Placebo	Alfacalcidol
Number at first enrollment	21	21
at final	19 (2 dropped out)	21
Age (yr)	70.60 ± 3.80	70.90 ± 4.00
Weight (kg)	61.28 ± 7.94	60.70 ± 6.66
Serum 25(OH)D_3 (ng/ml)	23.82 ± 3.24	24.71 ± 4.57
Serum iPTH (pg/ml)	43.86 ± 19.27	41.72 ±17.26
VDR genotype [n (%)]		
BB	-	2 (9.5%)
Bb	4 (19%)	5 (23.8%)
bb	17 (81%)	14 (66.7%)
Baseline muscle strength at 30°/sec (N-m)	19.20 ± 8.80	19.63 ± 6.10
Baseline muscle strength at 60°/sec (N-m)	18.01 ± 9.06	17.43 ± 7.19
Muscle strength at 12 wk, 30°/sec (N-m)	16.09 ± 5.28	20.28 ± 8.84
Muscle strength at 12 wk, 60°/sec (N-m)	15.05 ± 6.13	20.32 ± 8.56
Mean percent difference from baseline at 30°/sec (% ± SD)	-8.00 ± 29.90	3.17 ± 29.9*
Mean percent difference from baseline at 60°/sec (% ± SD)	-5.73 ± 46.5	27.13 ± 57.1*

Serum iPTH = serum intact parathyroid hormone, VDR = vitamin D receptor
* Improvement of muscle strength is defined as the mean percent difference of the muscle strength result at 12 weeks from the muscle strength at baseline ($p < 0.05$)

Table 4. ANCOVA table test of between-subject effects: the dependent variable is muscle strength at 12th week, 30°/sec

Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	1,149.977*	2	574.988	19.498	.000
Intercept	152.484	1	152.484	5.171	.029
MSB30	974.731	1	974.731	33.053	.000
DRG	160.261	1	160.261	5.434	.025
Error	1,091.139	37	29.490		
Total	15,622.080	40			
Corrected total	2,241.116	39			

* R Squared = 0.513 (adjusted R squared = 0.487),
MSB30 = baseline muscle strength at 30°/sec
DRG = alfacalcidol group significant improvement ($p = 0.025$)

Table 5. ANCOVA table test of between-subject effects: the dependent variable is muscle strength at 12th week, 60°/sec

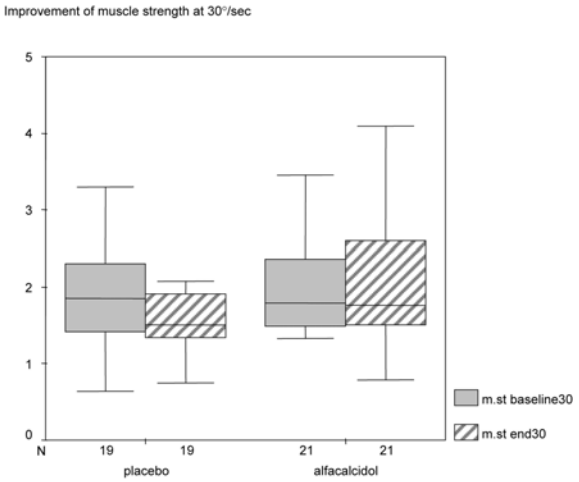
Source	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	1,271.603*	2	635.801	20.483	.000
Intercept	311.448	1	311.448	10.034	.003
MSB60	994.167	1	994.167	32.028	.000
DRG	338.184	1	338.184	10.895	.002
Error	1,148.486	37	31.040		
Total	15,120.403	40			
Corrected total	2,420.088	39			

* R Squared = 0.525 (adjusted R squared = 0.500),
MSB60 = baseline muscle strength at 60°/sec
DRG = alfacalcidol group significant improvement ($p = 0.002$)

most common side effect reported in the literature about the treatments of active vitamin D metabolites is hypercalcemia, but reports in trials using of alfacalcidol are rare. Two "drop-out" subjects: one had moderate dizziness after administration of intervention drugs; she intended to quit the drugs. The other sustained an ankle fracture and was discontinued in follow-up to this study. Both subjects were randomized to the placebo group, so there were not any significant adverse events in the treatment group.

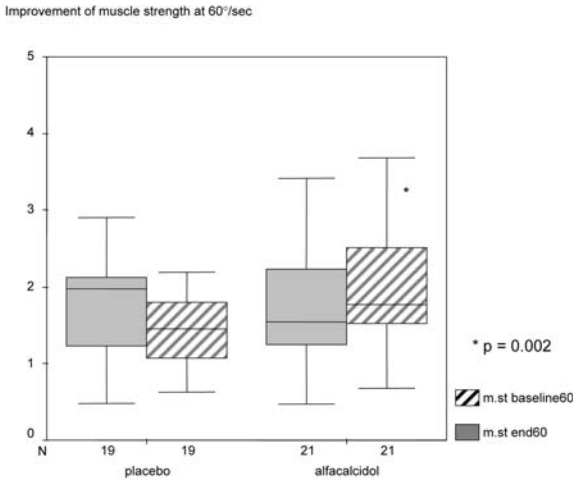
Discussion

In the clinical aspect, the relationship between vitamin D and the health status of adults or elderly



m.st baseline30 = muscle strength at baseline at 30°/sec,
m.st end30 = muscle strength at12 weeks at 30°/sec

Fig. 2 Improvement in muscle strength in the treatment group versus the placebo group in 30°/sec angular velocity



m.st baseline60 = muscle strength at baseline at 60°/sec,
m.st end60 = muscle strength at12 weeks at 60°/sec

Fig. 3 Improvement in muscle strength in the treatment group versus the placebo group in 60°/sec angular velocity

Table 6. Adverse events that potentially related to the drug investigation reported by subjects: number of cases (percent)

	Placebo	Alfacalcidol
Pruritus	1 (5.3%)	0
Dyspepsia	2 (10.5%)	1 (4.8%)
Mild headache	1 (5.3%)	1 (4.8%)
Mild dizziness	1 (5.3%)	0
Mild myalgia	0	1 (4.8%)

subjects had been largely ignored for a long time. The studies on vitamin D status of the elderly began only after mid-1970s when assays for serum $25(OH)D_3$ became available. Severe and prolonged deficiency in vitamin D is associated with osteomalacia characterized by defective mineralization of bone and decrease bone strength. On the other hand, hypovitaminosis D or vitamin D insufficiency that is not severe enough to cause osteomalacia, may contribute to hip fracture risk especially in the elderly. Although hip fractures among these elderly people may have several numbers of etiologies, the increasing in number of falls due to impaired muscle function is one of the most fascinating issues today.

Several studies have shown a high prevalence of hypovitaminosis D in the elderly population (30%-60%), especially in European countries[26,27]. In Thailand, as well as many countries in the topical zone, the debate points are still controversy. As mentioned earlier, one study[21] in Bangkok reported a normal level of serum $25(OH)D_3$ in 158 volunteers aged 20-80 while in Khon Kaen province, a study[22] on 106 elderly women the mean age of 69.4 years, reported a high prevalence of hypovitaminosis D. It is a problem of defining the term hypovitaminosis D which is still discussed on the clinically appropriate cut point. According to the study by Soontrapa et al[22], serum PTH concentration started to increase steeply when serum $25(OH)D_3$ concentration declined ≤ 30 ng/ml. By these criteria, hypovitaminosis D is defined as that population which serum $25(OH)D_3$ equal to or less than 30 ng/ml. This value corresponded to the study by Chapuy et al[26]. The serum level of PTH in their studied population increased when serum $25(OH)D_3$ value was lower than 31 ng/ml (78 nmol/L). In the present study, our percent of hypovitaminosis D was as high as 63.9%, if we used this cut-off point. This high prevalence of hypovitaminosis D in this study may be due to our population who were the outpatients usually coming to the hospital for some medical problems these women did not routinely exercise or walking outside their houses and because of their aging process which the synthesis of vitamin D are quite low.

It is well known that vitamin D is needed for strong bones when vitamn D is not sufficiency, the body cannot adequately absorb the calcium crucial to bone strength. What less well known is that vitamin D is also critical to proper muscle function. Striated muscles contain vitamin D receptors (VDR), and vitamin D has been shown to stimulate the synthesis of several important muscle proteins (troponin C, actin, Ca-ATPase) in the sarcoplasmic reticulum and inner membrane of mitochondria. In the study by Geusens et al[10], the statistically significant association between the VDR genotypes and quadriceps strength was observed only in non-obese, elderly women. A 23% difference in quadriceps strength between the two homozygote bb and BB genotypes were found. The distribution of these three genotypes in Asian population is not similar to the Caucasian. In our study, we have only a small number of BB genotype in the population. So we could not compare it with the baseline muscle strength as the study by Geusen et al[10] did.

For the primary outcome of this study, we have demonstrated that the muscle strength is significantly improved in the group that taken alfacalcidol compared with the group that had taken placebo. In the placebo group, only taken calcium carbonate cannot improve the muscle strength. On the other hand, the muscle strength decreases especially in the muscle strength at 60°/sec angular velocity which slightly more decreases than the one at30°/sec. The muscle strength at 60°/sec angular velocity is represent the strength in normal walking velocity in elderly people (60°- 90°/sec) while, the 30°/sec angular velocity is represented the strength in very slow walking velocity. The reasons why muscle strength deteriorated in the group that take only calcium are doubtful. One possibility is that intestinal absorption of calcium in these subjects is not good enough. Among various calcium compounds used as supplements, calcium carbonate is thought to be absorbed less efficiently than other calcium compounds, especially in the elderly people[28]. Since we do not allow all the subjects to take any vitamin supplements or any other calcium supplements during the study period, these women may develop subclinical hypocalcemia and some fatigue of muscle occurred. We did not measure the biochemical blood tests in the end of 12 weeks, so it may be mysterious to what happened to these subjects.

In our treatment group, the subjects who have received alfacalcidol and calcium carbonate significantly increase the muscle strength compared to the placebo in both angular velocities. The muscle strength at 60°/sec angular velocity has a much better increase in this aspect. As we know, the results of biopsy from vitamin D deficiency patients reveal selective atrophy of type-II muscle fibers with enlarged interfibrillar spaces and fat infiltration[29]. There are differences from neuropathic myopathy, which both type-I and type-II fibers were affected, and from immobilization myopathy, which type-I fibers were primary affected[30]. Sato et al[31] recently investigated muscle biopsies in hip fracture patients. The patients were divided into vitamin D-sufficient group ($25(OH)D_3$ > 39 nmol/L, n = 20) and vitamin D-deficient group ($25(OH)D_3$ < 39 nmol/L, n = 22). In the vitamin-deficient group, type-II fibers were significantly smaller (15.4 $\pm$ 4.2 mm) than in the vitamin D-sufficient group (38.7 $\pm$ 8.1 mm)($p < 0.0001$)[31]. Furthermore, in the vitamin D-deficient group, type-II muscle fiber diameters also correlated with serum levels of $25(OH)D_3$ ($r = 0.714$, $p = 0.001$)[31]. The muscle fibers which are responsible for fast and strong action are type-II muscle fibers. Study by Sato et al, clearly demonstrate that type-II muscle fibers were the main defect in vitamin D deficient myopathy. Theoretically, if we give vitamin D to those subjects, who have hypovitaminosis D, the muscle fibers that would respond better might be the type-II fibers. These correspond to our findings that muscle strength in 60º/sec angular velocity, which type-II muscle fibers are responsible, is actively react to the given or not given alfacalcidol.

Three possible mechanisms whereby vitamin D can improve muscle strength have been aimed. Firstly, we call the system as “calcemic action”. Vitamin D may act by controlling ionized calcium concentrations. As we know that calcium ions are the important factors in muscle contraction processes. Secondly, this mechanism may have a direct action on muscle cells on inducing expression of specific genes. Since we discovered the VDR in muscle cells, we have found many “genomic action” of vitamin D that induced expression of many proteins such as vitamin D binding protein, troponin C and actin. Finally, the “non-genomic action” which is rapid and mediated through membrane-bound receptors is the third mechanism. The binding to the receptor initiates a cascade leading to the formation of second messenger or phosphorylation of intracellular proteins.

Although our results correspond to the results of many studies[5,19,20] which demonstrated that supplement with vitamin D in the elderly population

could improved muscle strength. Some limitations of these studies were the sizes of samples. They were too small to demonstrate the acceptable prevalence of hypovitaminosis D and the significant correlation of VDR genotype to the muscle strength. Further studies to confirm our results in bigger sample sizes may be more properly conducted.

In conclusion, muscle strength declined in ageing of ambulatory elderly women. Hypovitaminosis D has a significant contribution to the fall and fractures in this elderly population, especially in those who already have osteoporotic bone. Our prevention and treatment regimens for osteoporotic elderly women especially in Thailand usually have no routine vitamin D supplementation. The results of our study, as well as of others have shown higher than expect of the percent of the hypovitaminosis D, and significant improvement of muscle strength in the elderly post-menopausal women having hypovitaminosis D after taking supplements with alfacalcidol. The routine administration of vitamin D or vitamin D metabolites to the regimen in prevention and treatment of osteoporosis, especially in elderly women, should be performed.

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การให้ยาแอลฟาแคลซิดอลร่วมกับแคลเซียมสามารถเพิ่มความแข็งแรงของกล้ามเนื้อ ควอดไดรเซพส์ในผู้หญิงสูงอายุไทยที่มีภาวะวิตามินดีพร่อง: การศึกษาแบบสุ่มมีกลุ่มควบคุม

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วัตถุประสงค์: *เพื่อประเมินผลการใช้ยาอัลฟาแคลซิดอลและแคลเซียม ในการเพิ่มความแข็งแรงของกล้ามเนื้อในผู้หญิงสูงอายุไทยที่มีอายุเท่ากับหรือมากกว่า 65 ปี ซึ่งมีภาวะวิตามินดีในเลือดพร่อง*

วัสดุและวิธีการ: *ผู้หญิงสูงอายุที่มีอายุเท่ากับหรือมากกว่า 65 ปี จำนวน 72 รายได้เข้าร่วมการศึกษานี้ ทั้งหมดนี้จะได้รับการเจาะเลือดเพื่อตรวจหาระดับ 25(OH)D_3 ฮอร์โมนพาราไทรอยด์ และชนิดของ vitamin D receptor genotypes หลังจากนั้นทั้งหมดจะได้รับการตรวจวัดความแข็งแรงของกล้ามเนื้อ quadriceps โดยใช้เครื่องมือ isokinetic dynamometer device มีผู้หญิงสูงอายุ 42 รายที่เข้าเกณฑ์การคัดเลือกและตกลงใจเข้าสู่การศึกษาแบบสุ่มเปรียบเทียบผลการรักษาโดยแบ่งเป็นสองกลุ่มคือ กลุ่มที่ได้ยาอัลฟาแคลซิดอล 0.5 มิลลิกรัม/วัน ร่วมกับยาเม็ดแคลเซียม 1,000 มิลลิกรัม/วัน กับกลุ่มที่ได้รับยาหลอกร่วมกับยาเม็ดแคลเซียม 1,000 มิลลิกรัม/วัน*

ผลการศึกษา: *หลังจาก 12 สัปดาห์ มีผู้หญิงสูงอายุ 40 รายเข้ารับการตรวจวัดความแข็งแรงของกล้ามเนื้อ quadriceps เป็นครั้งที่สอง จากการวิเคราะห์ด้วย ANCOVA พบว่ามีการเพิ่มขึ้นอย่างมีนัยสำคัญของความแข็งแรงของกล้ามเนื้อภายหลังได้รับยาอัลฟาแคลซิดอลเมื่อเทียบกับยาหลอก ทั้งความแข็งแรงกล้ามเนื้อที่ 30 องศา/วินาที (20.28 เทียบกับ 16.29, p = 0.025) และ 60 องศา/วินาที (20.32 เทียบกับ 15.05, p = 0.002)*

สรุป: *การใช้ยาอัลฟาแคลซิดอล 0.5 มิลลิกรัม/วัน ร่วมกับแคลเซียมสามารถเพิ่มความแข็งแรงของกล้ามเนื้อในผู้หญิงสูงอายุไทยที่มีอายุเท่ากับหรือมากกว่า 65 ปี ซึ่งมีภาวะวิตามินดีในเลือดพร่องได้ เมื่อเทียบกับการให้แคลเซียมอย่างเดียว*