

CHAPTER III

RESEARCH METHODOLOGY

3.1 Research Questions

3.1.1 Primary research question:

Can orally given alfacalcidol effectively improve quadriceps muscle strength in ambulatory elderly Thai women of 65 year of age or more, who have hypovitaminosis D, as measured by isokinetic dynamometer?

3.1.2 Secondary research question:

Are there any correlation between baseline levels of serum 25-hydroxyvitamin D, parathyroid hormone (PTH), and vitamin D receptor (VDR) gene polymorphism and the quadriceps muscle strength as measured by isokinetic dynamometer?

3.2 Research Objectives

1. To evaluate the efficacy of alfacalcidol 0.5 $\mu\text{g/d}$ plus calcium carbonate 1,500 mg/d compare to calcium carbonate 1,500 mg/d alone, on the improvement of muscle strength in ambulatory elderly Thai women in age group of 65 or more who have hypovitaminosis D (baseline serum $25(\text{OH})\text{D}_3 \leq 30 \text{ ng/ml}$), by measure the strength of quadriceps muscle as the indicators.
2. To evaluate the correlation between baseline serum 25 hydroxyvitamin D, parathyroid hormone (PTH) and vitamin D receptor (VDR) gene polymorphism to the baseline quadriceps muscle strength.

3.3 Research Hypothesis

Orally given alfacalcidol can improve quadriceps muscle strength, measured by isokinetic dynamometer, in ambulatory elderly Thai women in age group of 65 or more

Null hypothesis:

There are no difference between quadriceps muscle strength in treatment (alfacalcidol) group and control group, as measure by isokinetic dynamometer, after 12 weeks of intervention.

Alternative hypothesis:

There are a significant difference between quadriceps muscle strength in treatment (alfacalcidol) group and control group, as measure by isokinetic dynamometer, after 12 weeks of intervention.

$$H_0: \mu_T = \mu_C$$

$$H_a: \mu_T \neq \mu_C$$

Where :

μ_T = mean muscle strength in elderly Thai women ≥ 65 , after 12 weeks treated with alfacalcidol

μ_C = mean muscle strength in elderly Thai women ≥ 65 , after 12 weeks in placebo controlled group

3.4 Conceptual Framework

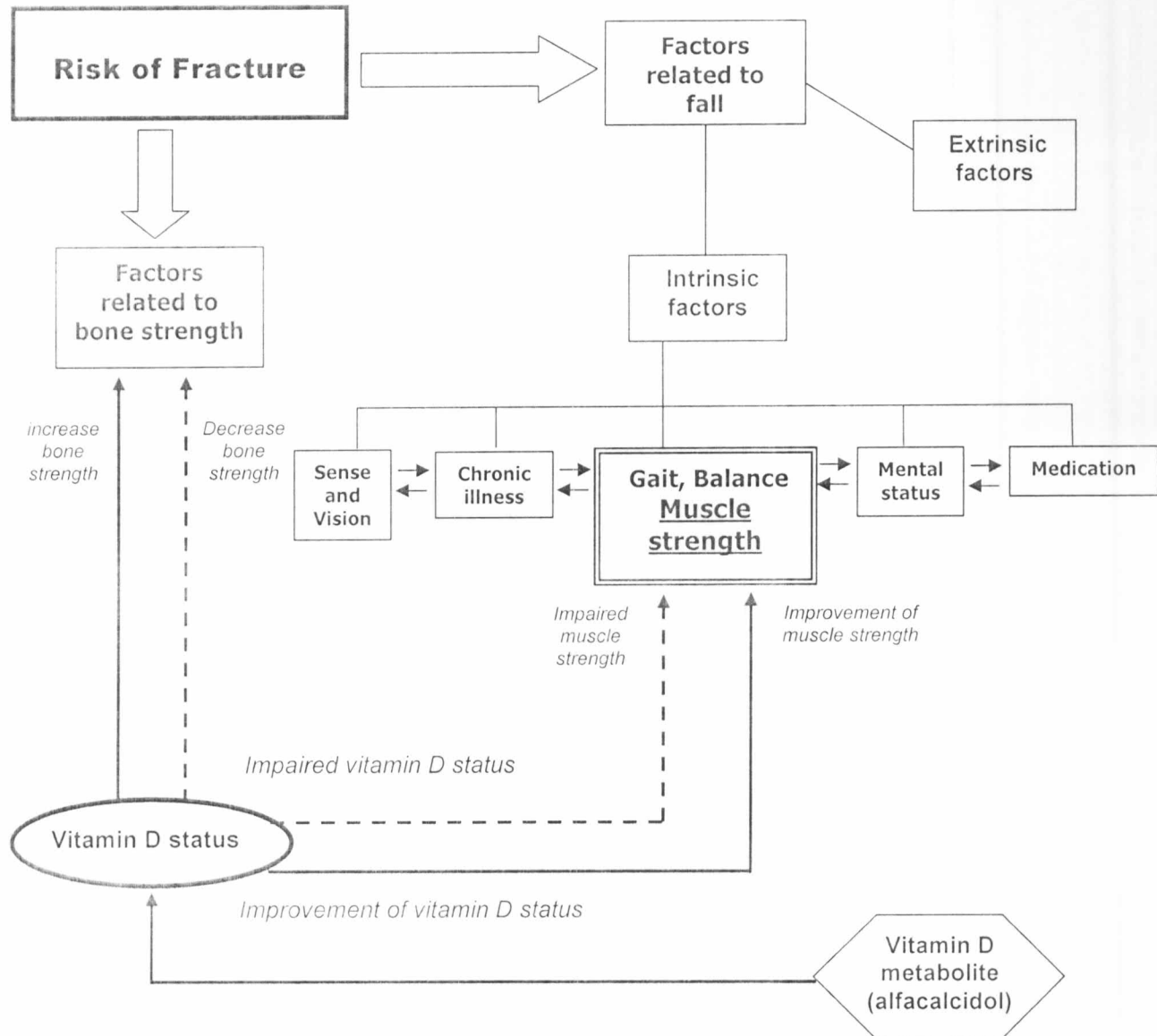


Figure 3.1 Conceptual framework demonstrates factors influencing hip fracture risks, vitamin D status is one of the risk factors for fracture known to influence both bone strength and risk of fall.

3.5 Operational Definition:

Vitamin D metabolites:

Derivative of vitamin D₃ (cholecalciferol) such as 25(OH) D₃ (Calcidiol) and 1,25(OH)₂ D₃ (Calcitriol) , which are active in our body. 1,25(OH)₂ D₃ are the most potent form of vitamin D and play as a substrate for the intracellular vitamin D receptor (VDR).

Alfacalcidol:

A synthetic vitamin D metabolite, which have formula as 1 α (OH) D₃. When administration into our body, it must undergo hydroxylation at liver to be 1,25(OH)₂ D₃ which is the hormonal active form.

25-hydroxyvitamin D₃ (25(OH) D₃):

The circulating form of vitamin D in our body, it used as the storage facility of vitamin D. Its concentration in serum is accepted to be the indicator for vitamin D status.

Hypovitaminosis D:

A level of serum vitamin D that influences calcium homeostasis and compensatory increase in PTH level. As in study by Soontrapa et al⁽³⁵⁾, it defined as a level of serum 25(OH) D₃ that equal or lower than 30 ng/ml (75 nmol/L). 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) D₃ and leads to osteomalacia and severely impaired muscle function.

Vitamin D receptor gene (VDR gene):

The gene encoded for vitamin D receptor (VDR), located on chromosome 12q and has several common allelic variants. One of the common and well-known restriction site polymorphism is Bsm1, which have three genotypes BB, Bb and bb. BB genotype of VDR is the most common genotype for the Caucasian, while bb genotype of VDR is the most common genotype of Asian peoples.

Parathyroid hormone (PTH):

One of major calcium regulating hormone, secreted from parathyroid gland. PTH and vitamin D interact in a number of complex ways. PTH stimulate the synthesis of $1,25(\text{OH})_2 \text{D}_3$ by kidney, in the other way, decrease in serum level of $1,25(\text{OH})_2 \text{D}_3$ can stimulate the synthesis and secretion of PTH from parathyroid gland.

3.6 Research Design:

This study was designed as a randomized double-blinded placebo-controlled experimental trial to answer the primary research question

A cross-sectional observational study, used correlation statistics was designed for answer the secondary research question. The study will find the relationship between the baseline serum $25(\text{OH}) \text{D}_3$, PTH and VDR genotype to the baseline muscle strength.

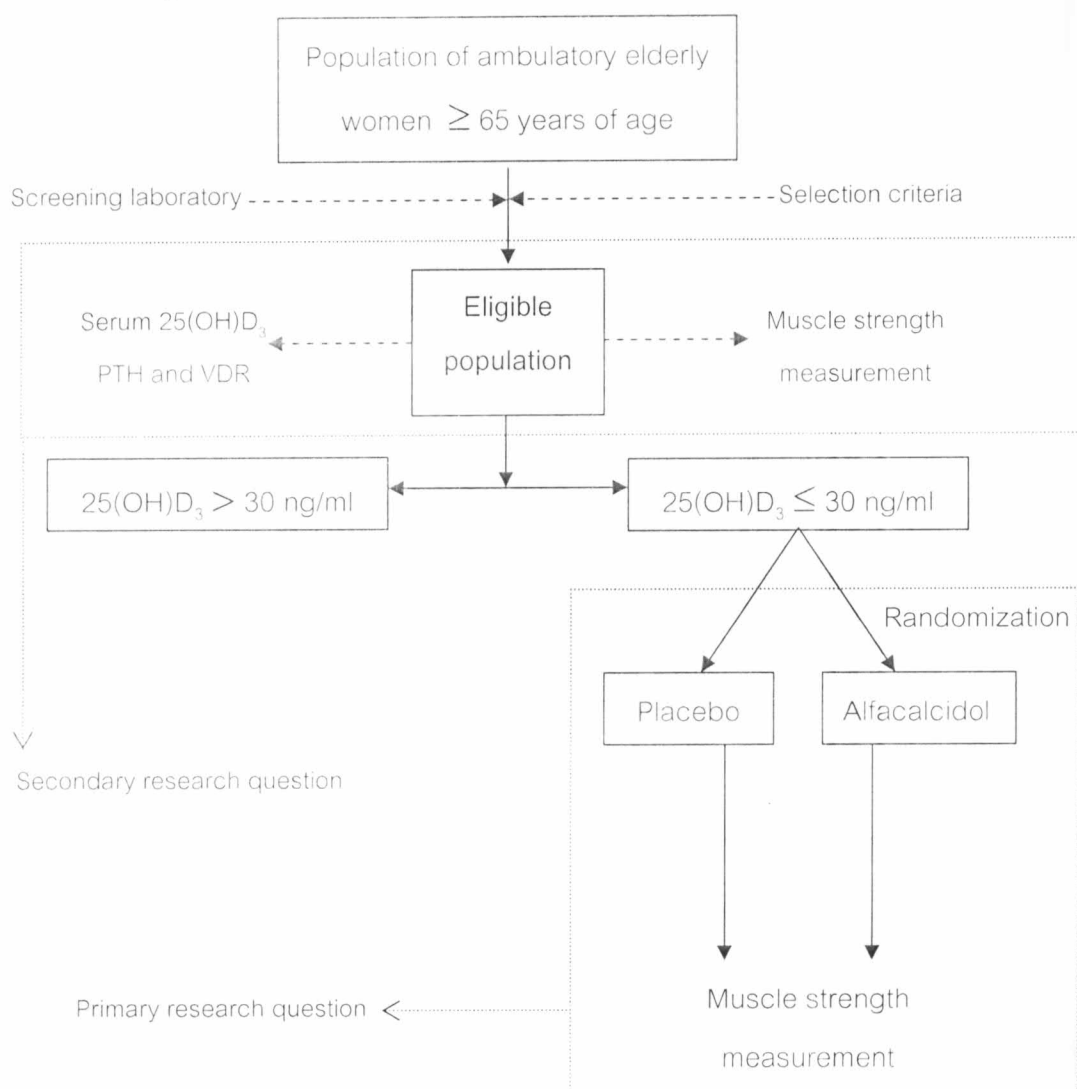


Figure 3.2 Diagram show research design algorithm

3.7 Population and Sample

3.7.1 Population

Target population:

Thai ambulatory elderly women 65 year of age or more

Sample population:

1. Those women who visit the osteoporosis clinic and geriatric clinic in Phramongkutkiao hospital during study period and fulfill the following criteria.
2. Those women who live in community or geriatric nursing home services, which fulfill the following criteria.

3.7.2 Sample selection

Eligible criteria:

Inclusion criteria:

- Thai elderly women aged 65 years or more
- Any race or ethnic group except Caucasian (because they may be some genetic different in response to vitamin D), living as citizen of Thailand.
- Eligible for blood testing and muscle strength testing
- Willingness to participate in the study, and ability to read and provide informed consent.

Exclusion criteria:

- History or evidence of metabolic bone diseases
- History of 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 that have major influence to the lower extremities function
- Congestive heart failure, coronary artery disease, angina, or myocardial infarction that have major influence to the muscle function testing
- Neurogenic diseases such as stroke, parkinsonism or dementia that have major influence to the muscle function testing

- Presence of cancer within 5 years.
- Evidence of significant renal (serum creatinine > 2.0 mg/dl) or liver impairment
- Visual abnormality which required major treatment or cannot corrected by eyeglass
- Prior use of fluoride, androgen, estrogen, calcitonin, or corticosteroids within 3 months
- Prior use of bisphosphonate within 6 months
- Prior use of investigation drug (vitamin D metabolites and calcium supplement) within 1 months
- Recently consumed an excess alcohol(>4 drink per day), or abused drugs

3.7.3 Sample size determination

Hypothesis testing for difference of two means

μ_T = mean muscle strength in elderly Thai women ≥ 65 , after 12 weeks treated with alfacalcidol

μ_C = mean muscle strength in elderly Thai women ≥ 65 , after 12 weeks in placebo controlled group

Hypothesis $H_0: \mu_T = \mu_C$

$H_a: \mu_T \neq \mu_C$

Sample size is calculated from this formula:

$$N = \frac{2\sigma^2 [Z_\alpha + Z_\beta]^2}{(\mu_1 - \mu_2)^2}$$

- According to the study of Aquino MA et al(39), mean isokinetic peak torque (PT) of knee extensor was 76.97 +/-14.59 Nm

- We expect the different between alfacalcidol and controlled group about 20%, which is the value of

Given that α error = 0.05 (two-tailed), $Z_\alpha = 1.96$

$$\beta_{\text{error}} = 0.2 \text{ (power 80\%), } Z_{\beta} = 0.84$$

$$N/\text{group} = 15$$

- Compensated for drop out of 20%, N /group are 18 cases

3.7.4 Allocation technique

At osteoporotic clinic, the ambulatory elderly women age of 65 years or more, who came to attend, were selected according to the inclusion and exclusion criteria. Women who consent for the study were included. After that, they were sent for blood testing and muscle strength testing by masked technicians.

After they came back, the elderly women who had serum 25(OH) D₃ ≤ 30 ng/ml were enrolled to the experimental randomized controlled trial (RCT). Those women were allocated to either treatment (alfacalcidol 0.5 µg/d + calcium carbonate 1,500 mg/d) and control (placebo + calcium carbonate 1,500 mg/d) groups by simple randomization. The allocation was concealed with the sealed, opaque envelope kept by the assistant nurse at the clinic.

For those elderly women whose serum 25(OH) D₃ > 30 ng/ml, their baseline data were kept for analysis of secondary outcomes.

3.8 Intervention:

Medication:

- Treatment group: Alfacalcidol 0.5 µg was administered for once daily in the morning after meal.
- Control group: Placebo, which have the same characteristic as active drug was administered for once daily in the morning after meal.
- For all subjects 1,500 mg of calcium carbonate tablet were provided immediately after dinner once daily.

We used the Alfacalcidol tablets and placebo tablets, which have the same characteristic as true active drugs. This supported by Teijin Co Ltd., Japan.

3.9 Outcome Measurements

Primary outcome

- Mean quadriceps muscle strength at 12 weeks in all groups

Secondary outcome

- Correlation between baseline serum 25 hydroxyvitamin D, PTH and VDR to the muscle strength at baseline.

- Percent of hypovitaminosis D in this study. (defined as those who serum 25(OH) D₃ ≤ 30 ng/ml)

- Occurrence and severity of adverse events.

3.10 Data Collection

The following data were recorded:

At baseline

- 1) Demographic data: age, weight
- 2) Blood testing:
 - Liver and kidney function test for screening
 - Serum 25(OH)D₃
 - Serum PTH
 - VDR genotype
- 3) Muscle strength measurements
 - Quadriceps muscle (present as average of both side)

Outcome:

- 1) Muscle strength measurements

Quadriceps muscle strength as measured as isokinetic peak torque at 12 weeks
- 2) Adverse events (AEs) : each AEs were recorded during the follow up period at 4 weeks, 8 weeks and 12 weeks after begin intervention.

3.11 Data Analysis

Table 3.1 Summary of measured variables

Data	type of variables	presentation
<i>Administrative variables:</i>		
- Name		
- Identification number		
- Telephone number		
<i>Demographic data:</i>		
- Age (yr)	continuous numerical	mean, SD
- Weight (kg)	continuous numerical	mean, SD
<i>Baseline variable :</i>		
- Blood testing for liver and kidney function	—— test for screening only ——	
- Serum 1,25(OH) ₂ D ₃ (ng/ml)	continuous numerical	mean, SD
- Serum iPTH (pg/ml)	continuous numerical	mean, SD
- VDR genotype	ordinal categorical	N (%)
- Muscle strength (N-m) (present as average of quadriceps muscle strength of both side)	continuous numerical	mean, SD
<i>Outcome variables:</i>		
- Muscle strength (N-m) (present as average of quadriceps muscle strength of both side)	continuous numerical	mean, SD
- Improvement of muscle strength (improvement of muscle strength = muscle strength at 12 weeks – muscle strength at baseline)	continuous numerical	mean, SD

Venous blood was collected from all participants for analysis of serum 25(OH)D₃, serum intact PTH and vitamin D receptor (VDR) genotypes. Serum 25(OH)D₃ 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).

After blood testing, all participants were sent to measure the quadriceps muscle strength using the isokinetic dynamometer device (Biodex, USA). Measurement was performed by a single physical therapist that had 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 $30^{\circ}/\text{sec}$ and $60^{\circ}/\text{sec}$ 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) expressed in Newton-meters (N-m). All measurements were done in both side (left and right) in each angle of velocity and used their average as a result of each subject, each angle. All values were managed as continuous numerical variables.

In each subject, measurement of all muscle strength variables were done in 2 times, before begin the intervention as the baseline data (together with blood testing), and at 12 weeks before intervention stopped. By using 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 placebo groups at 12 weeks.

About secondary outcomes of interest, we use blood testing at baseline for serum level of 25 hydroxyvitamin D, PTH and VDR gene phenotype. These data were use to find correlation with the muscle strength, using Pearson's correlation coefficient. The percent of hypovitaminosis D in this study was discussed in compared with the result of Soontrapa et al. (35). All data was analyzed on per protocol analytical basis.

All statistical analysis was performed using SPSS/PC version 11. A 2-sided significance level of 0.05 was used for analyses.

3.12 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 used of this drug is rarely report, because of this drug should have to metabolites at liver before become the active form.

The protocol must be reviewed and approved by the ethical committee of Phramongkutklao Army Hospital and King Chulalongkorn Memorial Hospital review board. All eligible subjects will receive detail of the study by research assistants, who will explain the protocol thoroughly about the following items:

- Detail protocol, objectives and methods of study.
- Treatment outcomes and potential adverse events.
- 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 were obtained from the subjects without enforcement. There was some ethical consideration about those control group, which received placebo. Usually those subjects had no significant symptom at all, and all of them were received calcium carbonate in dose of 1,500 mg/d for 12 weeks.

3.13 Limitation

There were some limitation in using the isokinetic dynamometer machine to measure the muscle strength, because of it needed the complicated techniques, it took time to measure, and it had limitation in only some university hospitals. In this study, the measurement of this parameter was performed in Department of Rehabilitation Medicine, Ramathibodi Hospital, by expert technician in this machine.

As we didn't know the exact prevalence of hypovitaminosis D in our Thai population, it was difficult to know the exact numbers of subject who had serum 25(OH)D \leq 30 ng/ml.

3.14 Expected benefit from this Study

Generalizability:

The result of this study could only applicable to those women in 65 years of age or more, and especially Thai women. But these were the group that high risk for hypovitaminosis D. Limited evidence about prevalence of hypovitaminosis D and their correlation to muscle strength, especially in Thai, so after this study, further bigger and multicenter study should be consider.

Expected benefits:

Subclinical type of vitamin D deficiency and hypovitaminosis D commonly found in associated with osteoporosis in elderly women. Reduced function of muscle units due to these conditions could result in increased frequency of falls, as one of risk factor leading to increased of osteoporotic non-vertebral fractures.

Our prevention and treatment regimens for osteoporotic women in Thailand usually have no routinely vitamin D supplementation. Because of the belief that there is adequate sunlight, which seemingly produced a sufficient vitamin D level in Thai people. If we can prove that it still have some population that have hypovitaminosis D, and they can improved with a treatment with vitamin D and calcium supplementation. Addition of vitamin D to the regimen in prevention of osteoporotic non-vertebral fractures especially in elderly women will routinely performed.