การเสริมวิตามินดีและสมรรถภาพทางกายในผู้ป่วยข้อเข่าเสื่อม

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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VITAMIN D SUPPLEMENTATION AND PHYSICAL PERFORMANCE IN KNEE OSTEOARTHRITIS PATIENTS

Miss Pacharee Manoy

A Dissertation Submitted in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy Program in Medical Science
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ปาจรีย์ มาน้อย : การเสริมวิตามินดีและสมรรถภาพทางกายในผู้ป่วยข้อเข่าเสื่อม (VITAMIN D SUPPLEMENTATION AND PHYSICAL PERFORMANCE IN KNEE OSTEOARTHRITIS PATIENTS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ศ. นพ. สิทธิศักดิ์ หรรษาเวก, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. ดร.วิไล อโนมะศิริ, รศ. นพ. พงศ์ศักดิ์ ยุกตะนันทน์, 134 หน้า.

โรคข้อเสื่อมเป็นสาเหตุของความผิดปกติของข้อเป็นอาการปวดที่พบได้บ่อย ระดับวิตามินดีต่ำร่วมกับโรคข้อเสื่อมมีความสัมพันธ์กับประสิทธิภาพของกล้ามเนื้อลดลง วัตถุประสงค์ของการศึกษานี้เพื่อประเมินผลของการเสริมวิตามินดีต่อมวลกล้ามเนื้อ ความแข็งแรงของกล้ามเนื้อ และสมรรถภาพทางกายในผู้ป่วยข้อเข่าเสื่อมที่มีระดับวิตามินดีต่ำ รวมถึงประเมินความชุกของการขาดวิตามินดีและภาวะกล้ามเนื้อถดถอย (Sarcopenia) ในผู้ป่วยข้อเข่าเสื่อม โดยมีผู้เข้าร่วมการวิจัยทั้งหมด 238 ราย ทำการตรวจประเมินสัดส่วนของร่างกาย ความแข็งแรงของกล้ามเนื้อ สมรรถภาพทางกาย กลุ่มอาการเมแทบอลิก ระดับวิตามินดี เลปติน อินเตอร์ลูคิน-6 ฮอร์โมนพาราไทรอยด์ และโปรตีนคาร์บอนิล ผลการศึกษาพบว่า ผู้ป่วยข้อเข่าเสื่อมมีภาวะพร่องวิตามินดีมากถึงร้อยละ 49.60 ภาวะขาดวิตามินดีร้อยละ 33.60 และระดับวิตามินดีปกติร้อยละ 16.80 นอกจากนี้ทำการประเมินความชุกของภาวะกล้ามเนื้อถดถอย โดยใช้เกณฑ์ของ Asian Working Group for Sarcopenia (AWGS) พบว่า ร้อยละ 10.10 มีภาวะกล้ามเนื้อถดถอย ส่วนร้อยละ 12.60 และ 77.40 มีภาวะเริ่มกล้ามเนื้อถดถอยและมวลกล้ามเนื้อปกติตามลำดับ ผู้ป่วยข้อเข่าเสื่อมที่มีภาวะขาดวิตามินดีมีอายุน้อยกว่ากลุ่มที่มีระดับวิตามินดีปกติ มีความแข็งแรงของกล้ามเนื้อเหยียดเข่าต่ำ มีระดับของสารเคมีในเลือด คือ ไขมันแอลดีแอล ไขมันไตรกลีเซอไรด์ ฮอร์โมนพาราไทรอยด์ อินเตอร์ลูคิน-6 และ โปรตีนคาร์บอนิล เพิ่มสูงกว่าผู้ป่วยกลุ่มระดับวิตามินดีปกติ แต่สมรรถภาพภาพทางกายทั้งสามกลุ่มไม่แตกต่างกัน

งานวิจัยในส่วนที่สอง ศึกษาประสิทธิผลของการเสริมวิตามินดีในผู้ป่วยข้อเข่าเสื่อมที่มีระดับวิตามินดีต่ำ (25(OH)D < 30 ng/ml) จำนวน 175 ราย ได้รับการเสริมวิตามินดี (ergocalciferol) ขนาด 40,000 IU ต่อสัปดาห์ เป็นระยะเวลา 6 เดือน ค่าเฉลี่ยของวิตามินดีก่อนได้รับการเสริมเท่ากับ 20.73 ng/ml หลังจากเสริมวิตามินดีครบเป็นระยะเวลา 6 เดือน ค่าเฉลี่ยของวิตามินดีเท่ากับ 32.14 ng/ml ค่าเฉลี่ยของ ไขมันแอลดีแอล โปรตีนคาร์บอนิล และฮอร์โมนพาราไทรอยด์ลดลง คุณภาพชีวิตและอาการปวดปรับตัวดีขึ้นจากค่าเริ่มต้น รวมถึงแรงบีบมือและสรรมภาพทางกายเพิ่มขึ้น โดยสรุปงานวิจัยนี้พบว่า การเสริมวิตามินดีในระยะเวลา 6 เดือน ช่วยลดภาวะเครียดจากออกซิเดชันของโปรตีนและอาการปวด เพิ่มคุณภาพชีวิต แรงบีบมือ และสมรรถภาพทางกายในผู้ป่วยข้อเข่าเสื่อม

สาขาวิชา วิทยาศาสตร์การแพทย์ ลายมือชื่อนิสิต .....

ปีการศึกษา 2559 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

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5574908030 : MAJOR MEDICAL SCIENCE
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PACHAREE MANOY: VITAMIN D SUPPLEMENTATION AND PHYSICAL PERFORMANCE IN KNEE OSTEOARTHRITIS PATIENTS. ADVISOR: PROF. SITTISAK HONSAWEK, M.D., Ph.D., CO-ADVISOR: ASSOC. PROF. WILAI ANOMASIRI, Ph.D., ASSOC. PROF. PONGSAK YUKTANANDANA, M.D., 134 pp.

Osteoarthritis (OA) is a common cause of musculoskeletal disability and pain worldwide. Low vitamin D status and OA were related with an accelerated decline in muscle quality. The purpose of this study was to determine the efficacy of vitamin D supplementation on muscle mass, muscle strength, and physical performance in knee OA patients with low vitamin D status. Firstly, this study aimed to evaluate the prevalence of vitamin D deficiency and sarcopenia in knee OA patients. A total of 238 knee OA patients were enrolled. Body composition, muscle strength, physical performance, metabolic profile, serum 25-hydroxyvitamin D (25(OH)D), leptin, interleukin-6 (IL-6), parathyroid hormone (PTH), and protein carbonyl were evaluated. Our study showed that knee OA patients had highly prevalent vitamin D insufficiency (49.60%), vitamin D deficiency (33.60%) and vitamin D sufficiency (16.80%). Moreover, the prevalence of sarcopenia in knee OA patients using the Asian Working Group for Sarcopenia (AWGS) criteria demonstrated that 10.10% was sarcopenia, 12.60% and 77.40% were pre-sarcopenia, and non-sarcopenia, respectively. Knee OA patients with vitamin D deficiency were younger, lower knee extension force, higher levels of low-density lipoprotein (LDL) cholesterol, triglyceride, PTH, IL-6, and protein carbonyls than vitamin D sufficiency group but physical performance was not different among three groups.

Secondly, we further determined the efficacy of vitamin D_2 supplementation in knee OA patients with low vitamin D status (< 30 ng/ml). One hundred seventy-five knee OA patients with low vitamin D status were received 40,000 IU vitamin D_2 (ergocalciferol) per week for 6 months. Baseline mean serum 25(OH)D in knee OA patients was 20.73 ng/ml. After vitamin D_2 supplementation for 6 months, mean serum 25(OH)D was increased to 32.14 ng/ml, while mean LDL cholesterol, protein carbonyl, and PTH were all significantly decreased. Quality of life (SF-12) and pain (visual analog scale) were both significantly improved from baseline. Knee OA patients demonstrated the significant increasing grip strength and improvement for all physical performance measurements after vitamin D_2 supplementation. In conclusion, vitamin D_2 supplementation for 6 months reduced oxidative protein damage and pain while improved quality of life, grip strength and physical performance in knee OA patients.

Field of Study: Medical Science
Academic Year: 2016

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Advisor's Signature .....
Co-Advisor's Signature .....
Co-Advisor's Signature .....

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LIST OF ABBREVIATIONS

Abbreviation	Full name
$1,25(OH)_2D$	1,25-dihydroxyvitamin D
25(OH)D	25-hydroxyvitamin D
6MWT	Six-Minute Walk test
ASM	Appendicular skeletal muscle mass
ASMI	Appendicular skeletal muscle mass index
AWGS	Asian Working Group for Sarcopenia
BIA	Bioimpedance analysis
BCA	Bicinchoninic acid
BMI	Body mass index
BSA	Bovine serum albumin
Ca	Calcium
DBP	Diastolic blood pressure
DNPH	Dinitrophenylhydrazine
DXA	Dual energy X-ray absorptiometry
EDTA	Ethylene diamine tetraacetic acid
EWGSOP	European Working Group on Sarcopenia in Older People
FBS	Fetal bovine serum
GdHCl	Guanidine Hydrochloride
HDL	High-density lipoprotein cholesterol
HOMA-IR	Homeostasis model assessment of insulin resistance
hs-CRP	High-sensitivity C-reactive protein
IL-6	Interleukin-6
JSN	Joint space narrowing score
KCl	Potassium chloride
KH_2PO_4	Potassium phosphate dibasic
LDL	Low-density lipoprotein cholesterol

MCS	Mental health composite scores
MET	Metabolic equivalent of task
MetS	Metabolic syndrome
Na_2HPO_4	Sodium phosphate dibasic
NaCl	Sodium chloride
NaF	Sodium fluoride
NF-κb	Nuclear factor-κB
OA	Osteoarthritis
OR	Odds ratio
PAQ-EJ	Physical activity questionnaire for elderly Japanese in Thai version
PBS	Phosphate buffer saline
PCS	Physical health composite scores
PTH	Parathyroid hormone
RANK	Receptor activator of nuclear factor-κB
RANKL	Receptor activator of nuclear factor-κB ligand
RTL	Relative telomere length
RXR	Retinoid X receptor
SBP	Systolic blood pressure
SF-12	12-Item short form health survey
SMI	Skeletal muscle index
STS	Sit to stand
TCA	Trichloroacetic acid
TMB	3,3',5,5'-Tetramethylbenzidine
TNF-α	Tumor necrosis factor-alpha
TUGT	Timed up and go test
VAS	Visual analogue scale
VDR	Intramyonuclear vitamin D receptor
WC	Waist circumference
WOMAC	Western Ontario and McMaster Universities Osteoarthritis Index

CHAPTER I

Introduction

1. Background and rationale

Osteoarthritis (OA) is the most common cause of musculoskeletal disability and pain worldwide. It is characterized by degradation of articular cartilage including changes in subchondral bone, osteophyte formation, joint space narrowing, and synovial inflammation (1). The symptoms of disease increase with age and involve mainly joint pain, the surrounding of knee muscles shortening and reduced range of motion that leads to severe pain and disability in later life (2). Approximately 10% of people aged over 55 years have symptomatic knee OA and disability (3). There are many risk factors leading to early knee structural changes in the healthy populations. Ageing, obesity, mechanical stress, gender, previous injury, muscle weakness, genetics, and vitamin D deficiency are associated with OA pathogenesis (4).

Recently, several studies have highlighted the correlation between vitamin D status and OA patients. In fact, vitamin D plays an important role on the protein synthesis of muscle fibers and muscle contractility regulation (5). The prevalence of vitamin D deficiency increases with age which was found approximately 70% in older Caucasians and Asians (6). Previous studies showed a low vitamin D level correlated with decreased muscle strength, difficulty in physical function, increased risk of falls, fracture, hospitalization and sarcopenia leading to frailty in the elderly (7-9).

Sarcopenia is a newly acknowledged geriatric syndrome described by an age-related decline of skeletal muscle mass plus low muscle strength or physical performance (10). The influence of imbalance between positive regulators and negative regulators cannot maintain muscle mass and muscle strength when the positive regulators are diminished and the negative regulators are increased. Particularly, obesity, sedentary lifestyle, chronic low grade inflammation, low vitamin D status and arthritis were associated with an accelerated decline in muscle quality (11). In OA patients, various pain-associated limited mobility and functional impairments can lead to decreased muscle strength and slowly progresses toward sarcopenia (12). In addition, OA co-exists frequently with vitamin D deficiency which

63% of primary knee OA patients had low vitamin D status (13). There was a high prevalence rate of vitamin D deficiency in patients with knee OA aged less than 60 years (14). Besides, lower levels of 25-hydroxyvitamin D (25(OH)D) were associated with greater knee pain and increased progression of radiographic OA (15) and poor quadriceps function (16).

Vitamin D supplementation is alternative treatment in the elderly who are at greater risk of vitamin D deficiency and physical disability. Several intervention studies have investigated the effects of vitamin D supplementation increase muscle strength, improve physical function, and decrease the risk of falls in the older with low level of vitamin D (17-19). The interventional studies reported vitamin D supplementation increased muscle strength in women after stroke (20) and increased both muscle fiber size and intramyonuclear vitamin D receptor (VDR) concentration in older women who are mobility-limited (21). However, previous studies have reported that vitamin D supplementation did not improve muscle strength or physical function. There was no improvement of muscle strength or physical performance in a group of healthy community-dwelling older men (22). The treatment of vitamin D_3 did not significantly improved physical performance in overweight and non-western immigrants (23).

For OA patients, vitamin D supplementation did not reduce knee pain or cartilage volume loss (24). On the other hand, vitamin D treatment in patients with knee OA has been reported to decrease pain and improve knee function using Western Ontario and McMaster Universities (WOMAC) and visual analog scale (VAS) assessment (13). However, the role of vitamin D supplementation in knee OA patients on physical performance and muscle strength is still unknown. Therefore, this study aimed to investigate the effects of vitamin D supplementation on muscle strength and physical performance in knee OA patients with low vitamin D status. In addition, the relationships between the change of biological domain such as insulin resistance, leptin, interleukin-6 (IL-6), protein carbonyls, and the achieved serum 25(OH)D level in 6 months were determined. The physiological and biological outcomes may provide valuable information on the effects of pharmacological intervention for treatment or prevention of physical disability in OA patients.

2. Research question

Primary question

1. What are the effects of vitamin D supplementation on muscle mass, muscle strength and physical performance in knee OA patients?
2. What are the effects of vitamin D supplementation on biomarkers such as metabolic risk factors, 25(OH)D, calcium and phosphorus homeostasis, inflammation, adipokine and oxidative stress in knee OA patients?

Secondary question

3. What is the prevalence of vitamin D deficiency and sarcopenia in knee OA patients?
4. What are the effects of vitamin D supplementation on pain, health-related quality of life in knee OA patients?

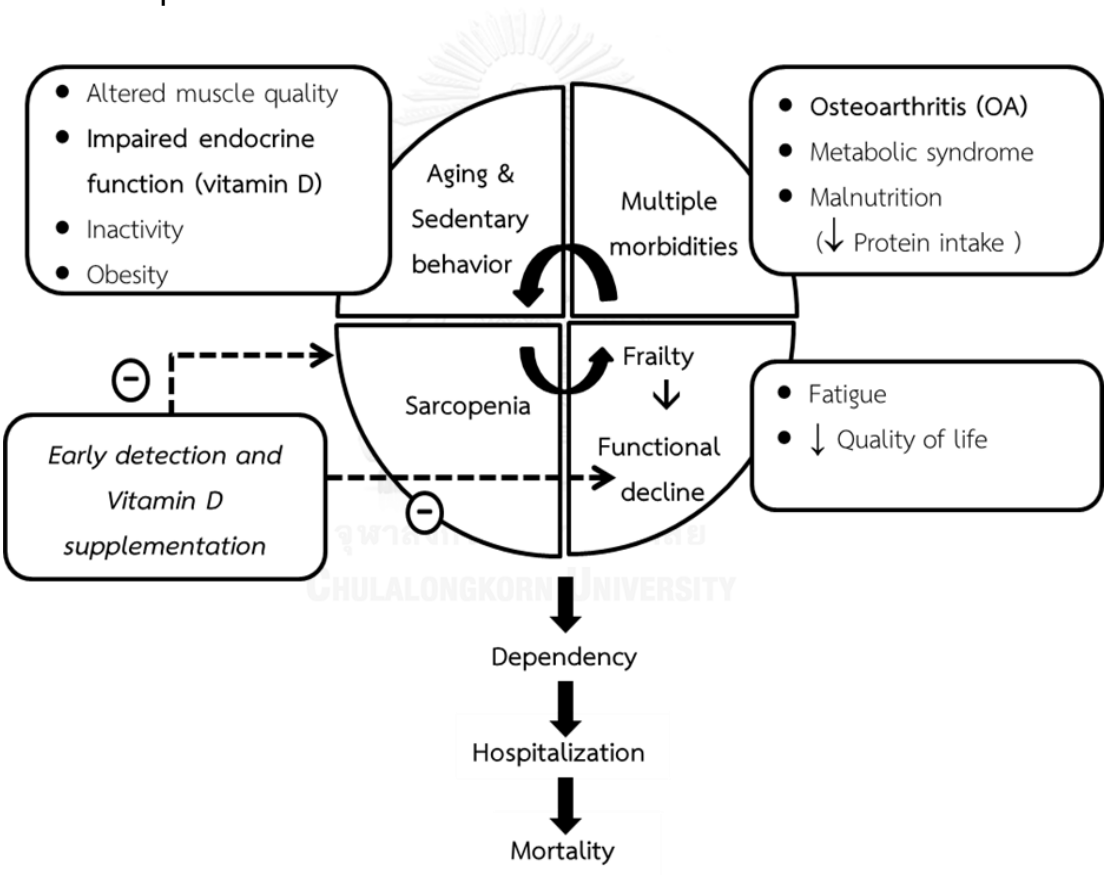
3. Objective

1. To determine the efficacy of vitamin D supplementation on muscle mass, muscle strength, and physical performance in knee OA patients with low vitamin D levels.
2. To evaluate the efficacy of vitamin D supplementation on the changes of biological markers levels such as metabolic risk factor, insulin resistance, 25(OH)D, calcium, phosphorus, PTH, IL-6, leptin, protein carbonyl and after vitamin D supplementation.
3. To evaluate the prevalence of vitamin D deficiency and sarcopenia in knee OA patients.
4. To evaluate the efficacy of vitamin D supplementation on pain and quality of life in knee OA patients.

4 Hypothesis

1. Vitamin D supplementation improve muscle strength, and physical performance in knee OA patients.
2. Vitamin D supplementation decrease levels of insulin resistance, PTH, leptin, IL-6, and protein carbonyls in knee OA patients.
3. Knee OA patients have decreased pain score and increased quality of life after vitamin D supplementation.

5. Conceptual framework



6. Key words

Vitamin D supplementation, Osteoarthritis, Sarcopenia, Muscle strength, Physical performance

7. Operational definition

- Osteoarthritis (OA) is defined as the symptom which is diagnosed by medical practitioners with sign at least three out of six of the following criteria such as age between 50-80 years old, morning stiffness lasting less than 30 minutes, crepitus on motion, bony tenderness, bony enlargement and no palpable warmth.
- Sarcopenia is a "newly acknowledged geriatric syndrome described by an age-related decline of skeletal muscle plus low muscle strength or physical performance".
- Metabolic syndrome (MetS) is presented if three or more of the following five criteria are met: waist circumference ≥90 cm in men or ≥80 cm in women; serum triglycerides ≥ 150 mg/dl; HDLC < 40 mg/dl in men or < 50 mg/dl in women; SBP ≥ 130 and/or DBP ≥ 85 mm Hg; and fasting blood glucose ≥ 100 mg/dl (25).

8. Ethical considerations

The study protocol was conducted according to the Belmont report by

1. Respect for persons

Participants had a right to decide for themselves whether to participate in research after receiving information about the research goals.

2. Beneficence/Non-Maleficence

Individuals with vitamin D insufficiency were eligible for vitamin D supplementation with 40,000 IU per week for 6 months.

3. Justice

The selection of research participants was recruited according to the inclusion and exclusion criteria of this study.

9. Expected benefit and application

1. Understand the effects of vitamin D supplementation on muscle mass, muscle strength and physical function in OA patient.

2. Understand the relationships of vitamin D and biological parameters in OA patients.

3. Application of vitamin D supplementation to prevent and/or delay onset of physical disability in OA patients.

10. Limitations

The subjects may drop out or lost follow up that it is difficult to collect the complete subjects in this study.

CHAPTER II

Literature Reviews

1. Osteoarthritis

Osteoarthritis (OA) is a common degenerative joint disorder in elderly people and is characterized by degeneration of articular cartilage, sclerosis in subchondral bone, osteophyte formation, and synovial inflammation. The clinical characteristics of OA are pain, swelling, stiffness, malformation of the joint, and loss of range of motion leading to limitation of physical function. Eighty percentage of OA patients with limited degree of movement experience impaired function in the workplace, and 25% of patients are unable to perform their main activities of daily life (ADL) which may result in social isolation and depression (26).

In recent years, however, it became clearer that osteoarthritis is not a simple degenerative process, systemic inflammatory and biomechanical factors are substantially involved in the pathogenesis and disease progression. An imbalance of catabolic and anabolic processes involve in tissue changes in OA. The cytokines of catabolic processes, particularly, interleukin-1β (IL-1β) and tumor-necrosis factor-α (TNF-α) activate production of proteolytic enzymes, such as matrix metalloproteinase (MMPs) that result in cartilage extracellular matrix damage. In addition, nitric oxide induces chondrocyte apoptosis which result in cartilage degradation. Articular cartilage has an important role in permitting low friction, absorption the transmitted forces during locomotion and providing joint stability (26, 27). Therefore, loss of cartilage, hypertrophy of the joint capsule, synovial inflammation, ligament laxity, joint pain and progressive muscle weakness to atrophy lead to physical disability in knee OA patients.

1.1 Diagnosis of knee OA

The most common symptom of OA is joint pain, especially increasing joint pain during weight-bearing activities. According to, the American College of Rheumatology (ACR) criteria, knee OA are classified using history, physical examination, radiographic and laboratory findings as shown in **Figure 2.1.**

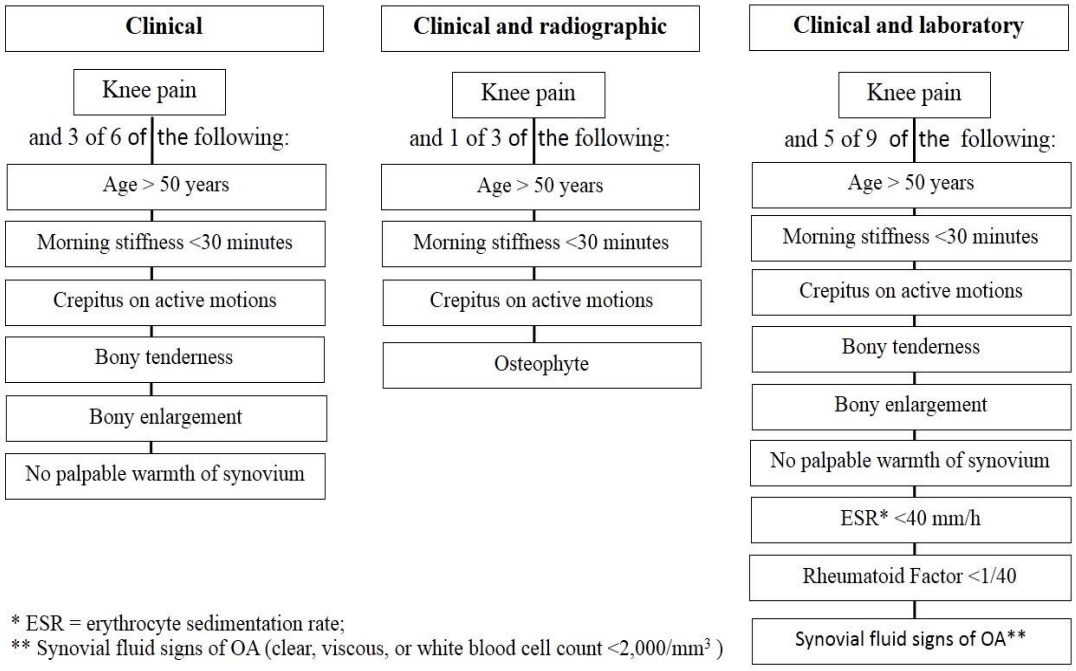


Figure 2.1 ACR criteria for the diagnosis of knee OA (28).

1.2 Risk factors for knee osteoarthritis

OA has a multifactorial etiologies which lead to early knee structural changes such as ageing, obesity, female sex, genetics, overuse, nutrition, mechanic factors, and previous injury (**Figure 2.2**).

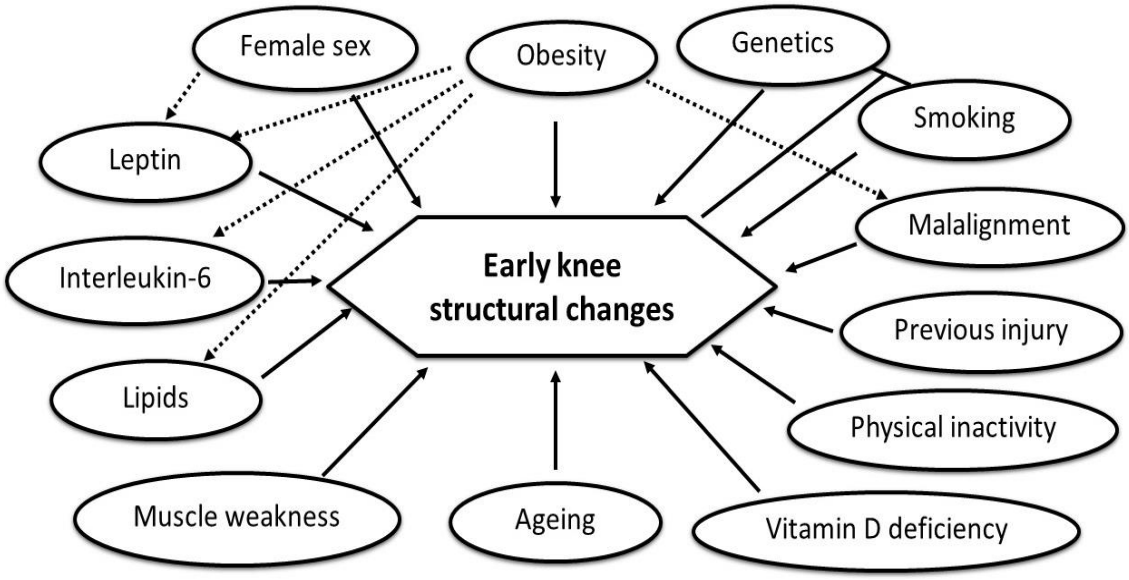


Figure 2.2 The risk factors associated with early knee structural changes (4).

1.2.1 Ageing

The symptomatic OA and radiographic evidence of OA increase with age. Ageing related with the risk of OA which include oxidative damage, decreasing cartilage volume, muscle weakness, and proprioception reduction. Moreover, basic cellular mechanisms for maintaining tissue homeostasis decrease with ageing. Accordingly, an inadequate response between catabolism and anabolism lead to joint tissue destruction and loss (29).

1.2.2 Gender

Females have higher risk of OA development than males. There are the multiple factors related with gender including hormonal influence effects on cartilage metabolism (estrogen), previous injury, malalignment (varus-valgus laxity) and muscle strength related with body weight (30).

1.2.3 Obesity and metabolic disease

Obesity is a major risk factor for the initiation and perpetuation of knee OA because a stress overload on weight-bearing joint would provoke cartilage damage. BMI>30 kg/m^2 was significantly associated with knee OA, with odds ratio (OR) of 2.81, and 95% CI of 1.32–5.96 (31). Moreover, present studies showed that OA is associated with metabolic syndrome (MetS), suggesting a possible pathogenic mechanism related with metabolic abnormalities and systemic inflammation. For instance, altered levels of proinflammatory cytokines and adipokines contribute to OA development by inducing the expression of proinflammatory factors and degradative enzymes that cause the inhibition of cartilage matrix synthesis and stimulation of subchondral bone turnover (32).

1.2.4 Muscle weakness

Muscle weakness related with knee OA that are the primary underlying cause of physical disability. Knee OA patients have quadriceps, hamstring and hip muscles weakness than age-matched healthy controls. The action of quadriceps and hamstrings provide a stability of the joint and shock absorption of tibiofemoral joint (33) Pervious study showed that quadriceps strength was related to the rate of lower extremity loading during walking in healthy females, the participants with quadriceps weakness had higher loading rates. Therefore, elevated joint loading rates may cause early-stage

knee OA (34). Moreover, muscle weakness interacts with low muscle mass that leads to sacopenia. It is likely that sacopenia is a cause of muscle atrophy inducing a decreased joint stability (35).

1.2.5 Genetic and epigenetic factors

Genetic and epigenetic factors appear to interplay with environmental factors and are involved in the development of primary OA. Many polymorphisms are associated with the change of human cartilage chondrocytes which may result in cartilage erosion (36).

1.2.6 Vitamin D deficiency

Vitamin D deficiency may play a role in the pathogenesis of OA on a clinical level (37) which 63% of primary knee OA patients had low vitamin D status (13). Accordingly, lower levels of 25-hydroxyvitamin D were associated with greater knee pain and increased progression of radiographic OA (15) and poor quadriceps function (16).

2. Osteoarthritis and sarcopenia

Sarcopenia is a syndrome characterized by progressive and generalized loss of skeletal muscle mass and function that occurs with advancing age. In 1989, Irwin Rosenberg reported the term of sarcopenia (Greek 'sarx' or flesh +'penia' or loss) to defined age-related reduce of muscle mass (10). Evidences presented the decline of skeletal muscle mass with ageing, which are 6% per decade between age 30 and 70 years in both sexes (38) and 1.4% per year after the age of 50. However, muscle mass and muscle strength began to reduce at early age of 35 years (39).

The adverse health outcomes of sarcopenia are physical disability, falls, hospital admission, poorer quality of life and mortality in older people (40) that features lead to frailty syndrome. A functional impairment may display to participate a core of the two conditions as shown in **Figure 2.3** (41). Consequently, it is important to search for a novel target of treatments for delaying onset disability or frailty in the elderly.

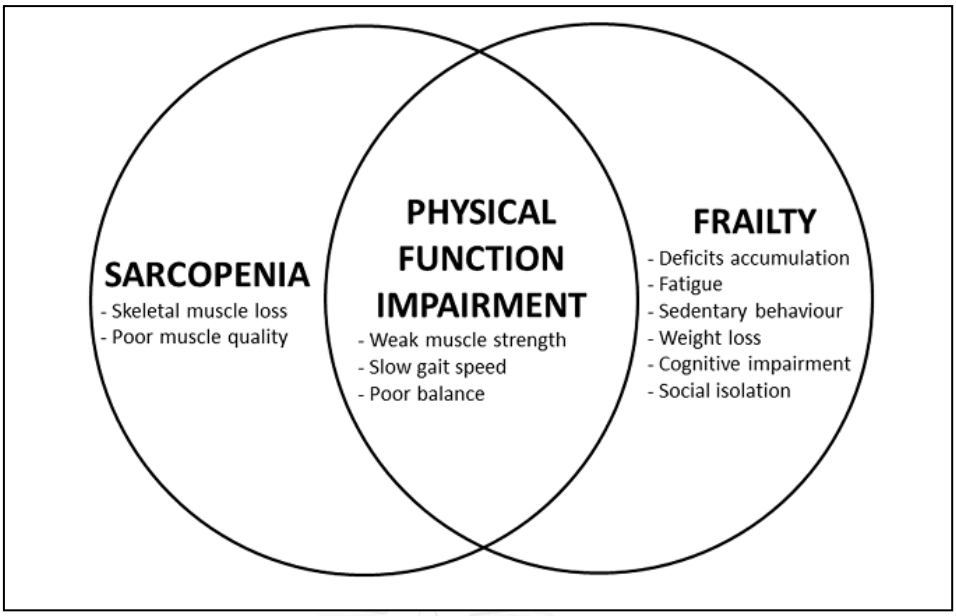


Figure 2.3 Association among sarcopenia, frailty, and physical function impairment (41).

2.1 Diagnosis of sarcopenia

There are several concepts regarding definition of sarcopenia. The European Working Group on Sarcopenia in Older People (EWGSOP) recommends using the presence of both low muscles mass and low muscle strength or physical performance for diagnosis sarcopenia as shown in **Table 2.1**

Table 2.1 Criteria for the diagnosis of sarcopenia (10)

Diagnosis is based on documentation of criterion 1 plus (criterion 2 or criterion 3)
1. Low muscle mass
2. Low muscle strength
3. Low physical performance

The cutoff points for sarcopenia

Sarcopenia was defined according to Asian Working Group for Sarcopenia (AWGS) (40). The characteristics of sarcopenia were defined by low muscle mass and low muscle strength or physical performance, whereas pre-sarcopenia was defined as only low muscle mass. The cutoff points were used as follows:

1) Muscle mass: SMI values based on lower than two SDs below the mean value of young reference groups was classified as low muscle mass. The cutoff value of SMI was <30.44 % in male and <25.81 % in female.

2) Grip strength: The cutoff value of grip strength was <26 kg in male and <18 kg in female.

3) Gait speed: The cutoff value of gait speed to be used was <0.8 m/s.

Stages of sarcopenia

EWGSOP suggests the severity of sarcopenia for guiding the clinical management and setting goals for treatments. Pre-sarcopenia is defined as only low muscle mass. Sarcopenia is characterised by low muscle mass and low muscle strength or physical performance, whereas, severe sarcopenia presents all three criteria as shown in **Table 2.2**.

Table 2.2 The stages of sarcopenia (10)

Stages	Muscle mass	Muscle strength		Physical performance
Pre-sarcopenia	↓			
Sarcopenia	↓	↓	Or	↓
Severe sarcopenia	↓	↓		↓

2.2 Measuring techniques for sarcopenia

Assessment techniques for sarcopenia in research and in clinical practice recommended by EWGSOP are shown in **Table 2.3**

Table 2.3 Assessments of muscle mass, strength, and physical performance for sarcopenia (10)

Variable	Research	Clinical practice
Muscle mass	- Computed tomography (CT) - Magnetic resonance imaging (MRI) - Dual energy X-ray absorptiometry (DXA) - Bioimpedance analysis (BIA) - Total or partial body potassium per fat-free soft tissue	- BIA - DXA - Anthropometry
Muscle strength	- Handgrip strength - Knee flexion/extension - Peak expiratory flow	- Handgrip strength
Physical performance	- Short Physical Performance Battery (SPPB) - Usual gait speed - Timed up-and-go test (TUGT) - Stair climb power test	- SPPB - Usual gait speed - TUGT

2.2.1 Muscle mass measurement

The assessment of muscle mass can be performed by using a wide range of techniques in order to estimating body composition, such as, dual energy X- ray absorptiometry (DXA), bioimpedance analysis (BIA) and anthropometry. The estimating skeletal muscle mass indices were measured *by* 2 methods, the first one is appendicular skeletal muscle mass index (ASMI) measured in terms of ASM/h^2 (ASM divided by square height). The second method is skeletal muscle index (SMI) measured in terms of percentage of ASM divided by body weight (ASM/W). The cutoff point for estimating low muscle mass using two SDs below the mean of muscle mass for the young reference group to define sarcopenia. In addition, the previous studies reported that sarcopenia defined in ASMI was associated with poorer physical performance (42)., SMI by the weight- adjusted skeletal muscle index was closely associated with metabolic syndrome (43), higher body mass index, gout, lack of regular exercise, and a history of falls that it can be used to identify subjects with sarcopenia (44).

2.2.2 Muscle strength measurement

Muscle strength is a major component to the assessment of sarcopenia and muscle quality. Several techniques are available for the assessment of muscle strength including isometric dynamometers and isokinetic muscle strength measures of power and torque (45). Handgrip strength is measured with a hand-held dynamometer with related with lower extremity muscle power, knee extension torque, and calf cross-sectional muscle area (46). Therefore, grip strength is suggested as a good indicator of muscle strength measurement. EWGSOP recommend the cut-off point low handgrip strength as < 30 kg for male and < 20 kg for female, whereas Asian Working Group for Sarcopenia (AWGS) suggests the cut-off point as < 26 kg for male and < 18 kg for female (40).

2.2.3 Physical performance measurement

There are several methods to assess physical performance. Gait speed is a highly reliable, simple, quick test, and inexpensive screening tool which indicates health related quality of life (47). EWGSOP and AWGS suggest gait speed $\leq$ 0.8 m/s for identifying the risk for sarcopenia (10, 40)

2.3 The prevalence of sarcopenia

Several studies identified sacopenia by muscle mass index, the prevalence of sarcopenia by using BIA found in the elderly aged ≥ 60 years was 7% in males and 10% in females (48), whereas the prevalence of sarcopenia by using DXA was 6 to 15% among the elderly aged ≥ 65 years (49). In Asia, the prevalence of sarcopenia based on EWGSOP criteria was 11.3% in males and 10.7 % in females (50). In Thailand, the prevalence of sarcopenia based on only DXA was 15.68 % and 12.9%, respectively, for males and females aged over 50 years (51).

Lee et al. classified osteoarthritis patients into four categories of body composition by using only DXA, which include normal, obesity, sarcopenia, and sarcopenic obesity. They found that the prevalence of sarcopenia and sarcopenic obesity was 4.3% and 3.0% respectively (52).

2.4 The pathophysiology of sarcopenia

The major risk factors of sarcopenia include neuromuscular aging, reduction in anabolic hormone, dysregulation of cytokine secretion, modification in the inflammatory state and chronic diseases. The imbalance of positive and negative regulators cannot maintain muscle mass, strength, and muscle functions when the positive regulators are diminished such as low vitamin D status and the negative regulators are increased, including inflammatory conditions. Moreover, the other factors, particularly, obesity, sedentary lifestyle, chronic low grade inflammation, and arthritis were associated with an accelerated decline in muscle quality leading to sarcopenia. (11) (**Figure 2.4**).

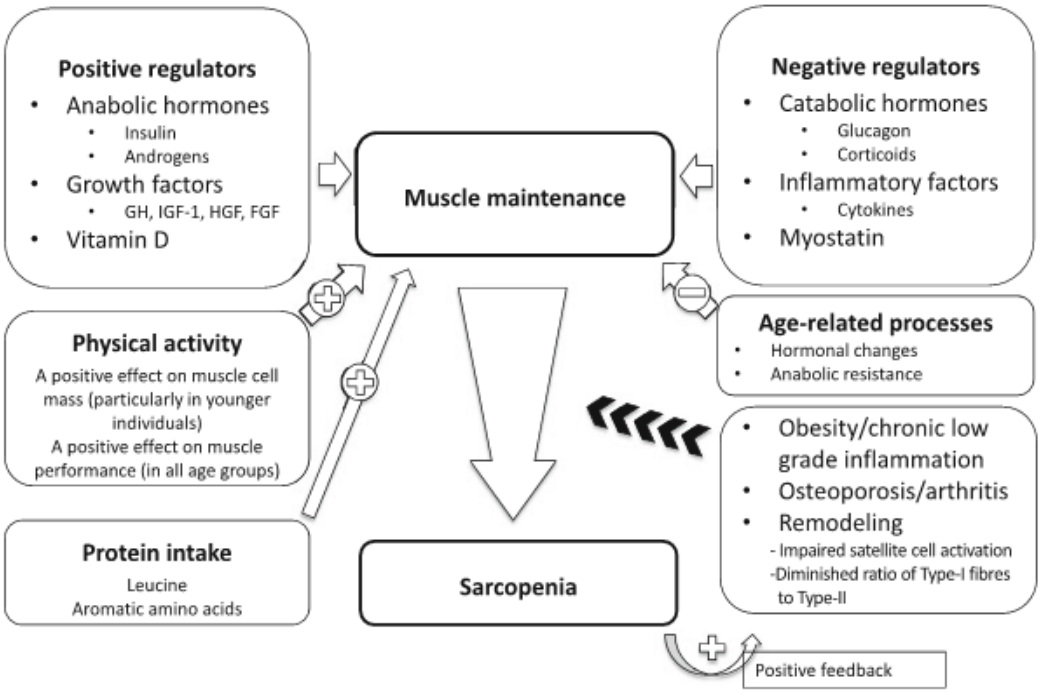


Figure 2.4 The main processes involved in the maintenance of muscle tissue and the decline toward sarcopenia (11)

3. Osteoarthritis and Obesity

Obesity is a complex syndrome which stimulates an abnormal neuroendocrine and pro-inflammatory pathways resulting in altered regulation of food intake, increased fat mass and alterations in energy metabolism (53). Moreover, obesity is linked to sarcopenia including increased adipocyte size and fat infiltration in muscle tissues and increased number of macrophages, inflammatory cytokines, reactive oxygen species, and insulin resistance related with physical disability (54).

3.1 Anthropometry

Cause of functional decline in OA relates with the changes in body composition that occurs with aging. Higher BMI was associated with an alteration of structure of meniscal and cartilage abnormalities leading to an increase progressive OA (55). In addition, obese or overweight individuals have high waist circumference which is one of five criteria of the MetS and associated with poor quality of life (56).

3.2 Metabolic syndrome (MetS)

Obesity increased risk of MetS. In 2005, International Diabetes Federation (IDF). using three or more of five of the following factors recognized as having MetS: central obesity (WC ≥90 cm in male and ≥80 cm in female), increased fasting blood glucose ≥100 mg/dl or under treatment for diabetes, decreased HDL cholesterol (≤40 mg/dL in male, ≤50 mg/dL in female or drug treatment), elevated triglycerides (≥150 mg/dL or drug treatment) and increased blood pressure ≥130/85 mmHg or drug treatment for hypertension (25).

3.3 Insulin resistance

Insulin resistance is one of the factors leading to the loss of skeletal muscle. Insulin is a peptide hormone synthesized in the ß cells of the pancreatic islets of Langerhans. Insulin regulates glucose homoeostasis by facilitating cellular glucose uptake, controlling carbohydrate, lipid and protein metabolism as well as stimulating cell division and growth through its mitogenic effects. Conversely, insulin resistance is defined as an impaired ability of target tissues to glucose disposal from the circulation (57). Normally, insulin provokes glucose transport protein type 4 (GLUT4) to transport glucose out of the bloodstream into skeletal muscle. It stimulates glycogen synthesis and lipid oxidation, releases amino acids for protein synthesis and suppresses proteolysis (**Figures 2.5 A**). When insulin-stimulated glucose transport into skeletal muscle is diminished, this results in inability to keep blood glucose concentrations within normal ranges. Besides, insulin resistance is associated with high levels of stored lipids in skeletal muscle cells, impaired insulin signaling, glycogen and protein synthesis (**Figures 2.5 B**) (57-59).

Insulin resistance in obesity may promote muscle catabolism, previous studies showed that insulin resistance was associated with low muscle mass and poor muscle strength. Sayer et al. reported that decreased grip strength was significantly associated with homeostasis model assessment of insulin resistance (HOMA-IR) as well as with increased odds ratio of having metabolic syndrome (60). Kim et al. found that insulin resistance, inflammation, and vitamin D deficiency were associated with sarcopenic

obesity (61). Chung et al reported that sarcopenia with OA had a higher OR of MetS and insulin resistance than those with OA or only sarcopenia (62). However, further research is needed to better explain the mechanisms underlying sarcopenia in order to prevent these conditions.

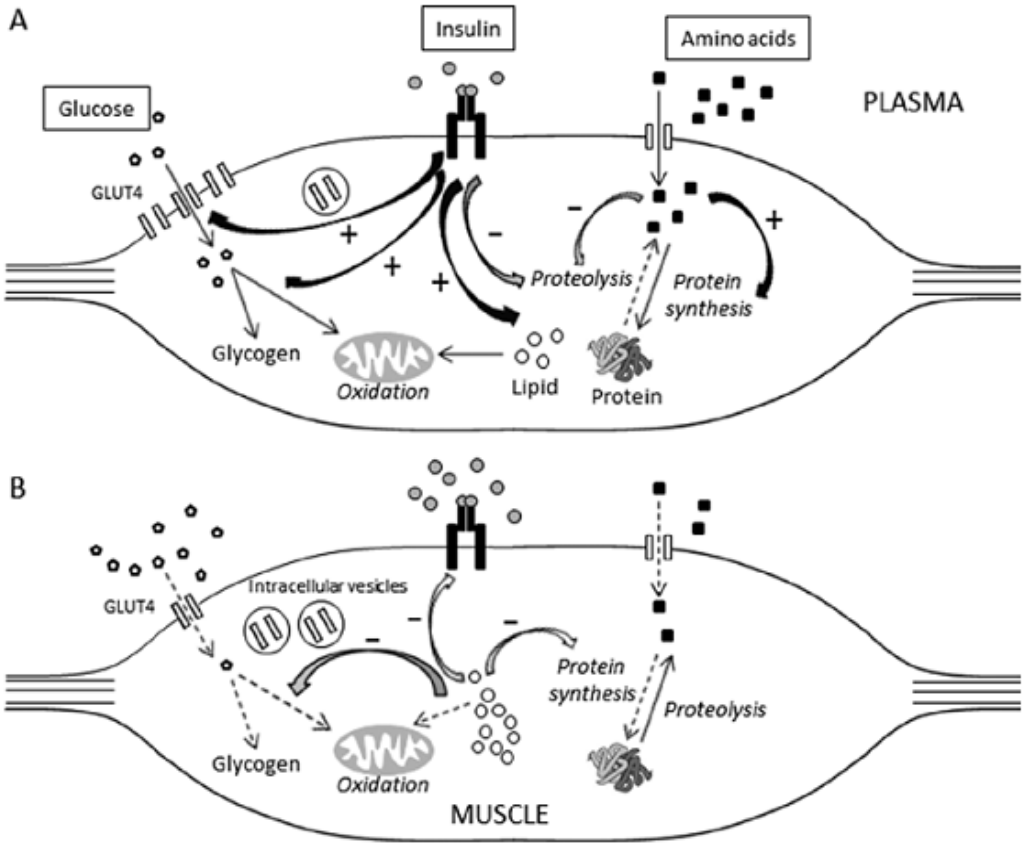


Figure 2.5 Insulin resistance in skeletal muscle: **A)** Normal muscle of young adult **B)** Muscle of elderly with sarcopenic obesity**.** (Straight arrows: metabolite flux. Broken straight arrows: reduced metabolite flux. Filled curved arrows: stimulatory effect. Open curved arrows: inhibitory effect) (58).

4. Osteoarthritis, inflammation, adipokine and oxidative stress

4.1 Inflammation: High-sensitivity C-reactive protein and Interleukin 6

4.1.1 High-sensitivity C-reactive protein (hs-CRP)

Hs-CRP is a sensitive marker of low grade systemic inflammation that response to inflammation, infection, and tissue damage (63). Hs-CRP is synthesized by hepatocytes in the liver and adipocytes and controlled by pro-inflammatory cytokines (64).

Pearle et al. found that OA patients with synovial inflammation had higher levels of hs-CRP and IL-6 than no inflammation (65). Moreover, hs-CRP levels are associated with severity of pain which measured by visual analogue scale (66).

4.1.2 Interleukin 6 (IL-6)

IL-6 is considered as a cytokine that strongly activates in immune system, hematopoiesis, and inflammation. It is produced substantially by many different cell types, including monocytes, macrophages, fibroblasts, T cells, B cells and endothelial cells (67). IL-6 is acknowledged as the "geriatric cytokine" because its levels elevate during aging process (68). High levels of IL-6, IL-1, TNF-α, and C-reactive protein (CRP) are related in elderly with high risk of morbidity and mortality. In muscle metabolism, IL-6 and TNF-α promote not only decreasing the production of myofilament proteins but also increasing oxidative stress and muscle catabolism (69).

Pereira et al. demonstrated that IL-6 had an inverse correlation with hand grip strength in elderly women residing in the community (70). In OA patients, there was an inverse association between levels of IL-6 and the peak torque/body mass value of hamstring/quadriceps muscle balance but there was no correlation between IL-6 levels and physical function by WOMAC measurement (71). Therefore, high levels of cytokine may or may not lead to muscle weakness.

4.2 Adipokine: Leptin

Adipose tissue is an active metabolic tissue that synthesizes hormones and proteins. Leptin is 16 kD a protein, secreted primarily by adipocytes from the white

adipose tissue that is encoded by the obese (ob) gene. Leptin acts through the leptin receptors (OB-Rb) which is the class 1 cytokine receptor superfamily. Therefore, both leptin and its receptor have structural and functional similarity to the interleukin-6. Leptin activates an intracellular signaling through nuclear factor kB (NF-kB), janus kinase (JAK) and signal transducers and activators of transcription (STAT) pathways leading to inflammation and angiogenesis (72, 73)

Leptin plays an active role in the regulation of energy balance by reducing food intake and increasing energy expenditure at the hypothalamic level. In obesity, leptin levels enhance in the circulation which present unsuccessfully to control food intake and body weight due to leptin resistance (73). Previous studies demonstrated that leptin had a positive correlation with body mass index (BMI) that obese individuals had significantly higher circulating leptin levels in comparison to non-obese people (74). Specifically, higher leptin levels were associated with sarcopenic visceral obesity (75) and poorer mobility-based functioning in middle-aged to the elderly (76).

In OA, the potential role of leptin is supported by synovial fluid leptin levels which were related positively with BMI (77) and severity of OA (78). Several studies indicated that leptin was associated with increasing levels of IL-1, IL-6, interferon γ, TNF-α, matrix metalloproteinases, and nitric oxide synthase type 2 (NOS_2) (79, 80). However, few studies had investigated leptin, muscle mass and physical performance in knee OA patients.

4.3 Oxidative stress: Protein carbonyls

Oxidative stress is one of factors leading to aging, degenerative diseases, atherosclerosis, and skeletal muscle dysfunction. Actually, oxidative stress occurs simultaneously when the formation of reactive oxygen species (ROS) increases, or when ROS are scavenging or the decreased repair of oxidative modification molecules occur. Typically, ROS present in the form of the superoxide anion ($O_2^{-\cdot}$), hydroxyl radicals (OH^-), and hydrogen peroxide (H_2O_2). Therefore, excess ROS cause oxidative damage to nucleic acids, proteins, and lipid (81-83).

Protein carbonyls are markers of oxidative damage to proteins leading to cellular dysfunction and a decline in tissue function. The direct oxidation of amino acids, such as lysine, arginine, histidine, proline, glutamic acid, and threonine, or by the binding of aldehydes or ketones can be reacted by 2,4-dinitrophenylhydrazine (DNPH) to form hydrazones that can be detected by spectrophotometry techniques (82) (**Figure 2.6**).

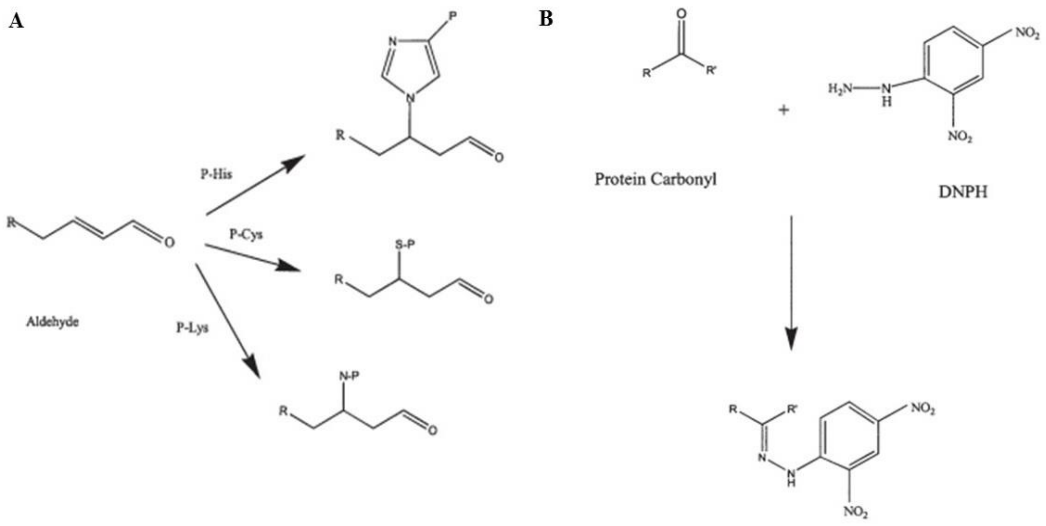


Figure 2.6 A) The structures of carbonyl group are produced by Michael addition: protein lysine (P-lys), histidine (P-His), or cysteine (P-Cys) residues **B)** The reaction between protein carbonyl groups and dinitrophenylhydrazine (DNPH) produced 2,4 –dinitrophenyl (DNP) hydrazone (82)

In aging, the levels of oxidative stress in skeletal muscle have been increased both at rest and during disuse suggesting that oxidative stress has a role in mediating disuse-induced and associated muscle loss. Semba et al. studied the relationship between serum protein carbonyl concentrations and walking speed among older women living in the community. They showed that protein carbonyl was related with low speed walking and the development of severe walking disability in older women (84). Howard et al. reported that high serum protein carbonyls were related with poor grip strength among disabled older women living in the community (85). Therefore,

increasing oxidative stress may contribute to loss of muscle strength and physical function in older adults.

5. Osteoarthritis and vitamin D

Vitamin D is a group of fat-soluble compounds that play an important role in calcium homeostasis and maintenance of normal bone metabolism through a negative feedback with the parathyroid hormone (86). Moreover, vitamin D is known as an essential factor in muscle metabolic processes by the protein synthesis of muscle fibers and regulating muscle contractility (5, 87). Previous study showed serum 25(OH)D less than 30 ng/ml was related with balance problems, impaired lower extremity function, high fall rates, low bone mineral density (BMD), and muscle weakness (88). Park et al. demonstrated that the risk of sarcopenia was increased by 1.46-fold by lowering serum 25 (OH)D by 10 ng/ml only in women (8). However, Marantes et al. found that serum 25(OH)D and parathyroid hormone (PTH) was not associated with skeletal muscle mass or strength, while women aged less than 65 years had an association between lower 25(OH)D levels and muscle mass (89).

5.1 Vitamin D metabolism

Vitamin D has two forms: ergocalciferol (activated ergosterol, vitamin D_2), found in plants and irradiated yeast; and cholecalciferol (activated 7-dehydrocholesterol, vitamin D_3), formed in human skin after exposure to ultraviolet B (UVB) rays from the sunlight. Vitamin D_3 binds to vitamin-D-binding protein (DBP) in the bloodstream and is transported to the liver where it is hydroxylated by liver 25-hydroxylases. While 1,25-dihydroxyvitamin D ($1,25(OH)_2D$), the active form of vitamin D in the kidney is activated by 1α-hydroxylase enzyme. The tight regulation of 1α-hydroxylase enzyme and activity in kidney including parathyroid hormone (PTH), calcium, fibroblast growth factors 23 (FGF23), and $1,25(OH)_2D$ levels. Particularly, $1,25(OH)_2D$ acts on various target tissues by vitamin D receptor (VDR) that $1,25(OH)_2D$ appears to affect both classical target and non-classical target tissues such as skeletal muscle possibly via the VDR. When, $1,25(OH)_2D$ induce the expression of 25-hydroxyvitamin D-24-hydroxylase

(24-OHase) to catabolize 1,25$(OH)_2$D to inactive metabolites, especially calcitric acid which it is excreted in the bile as presented in **Figure 2.7** (86, 90).

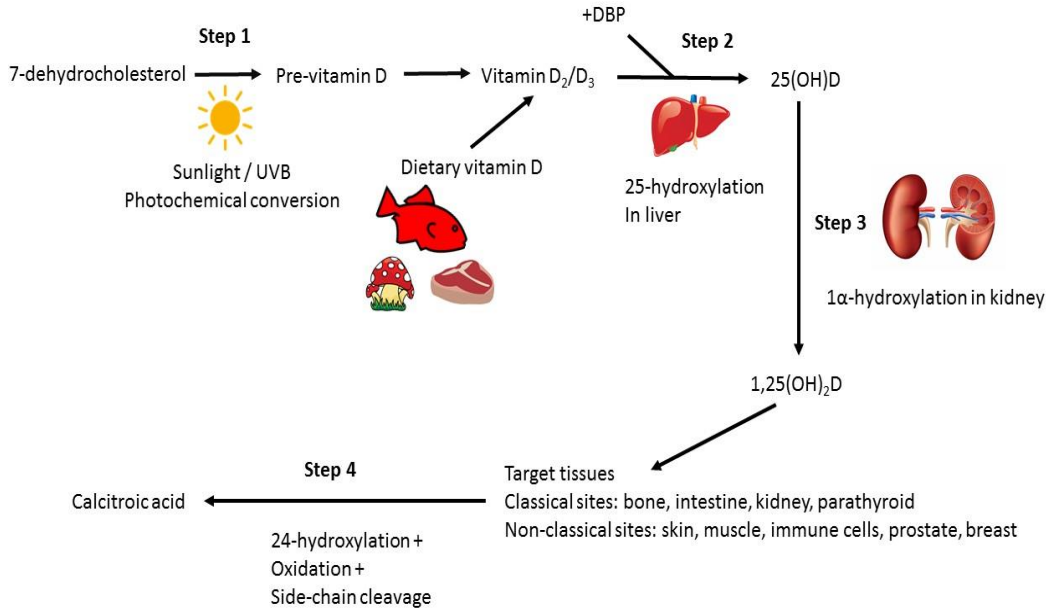


Figure 2.7 Vitamin D pathway (90).

5.2 Vitamin D mechanism of action

The biologically active form 1,25$(OH)_2$D binds VDR and acts as a ligand-activated transcription factor. VDR is a transcription factor and a member of the steroid hormone nuclear receptor family and consists of three domains: N-terminal dual zinc finger domain which bind to the grooves of the DNA, the C-terminal ligand that binds to 1,25$(OH)_2$D, and the hinge region that links these two domains together. Therefore, the steps for the control of gene transcription involve 1) ligand binding, 2) heterodimerization with retinoid X receptor (RXR), 3) binding of the heterodimer to vitamin D response elements (VDRE), and 4) recruitment of other nuclear proteins into the transcriptional preinitiation complex (90, 91) (**Figure 2.8**).

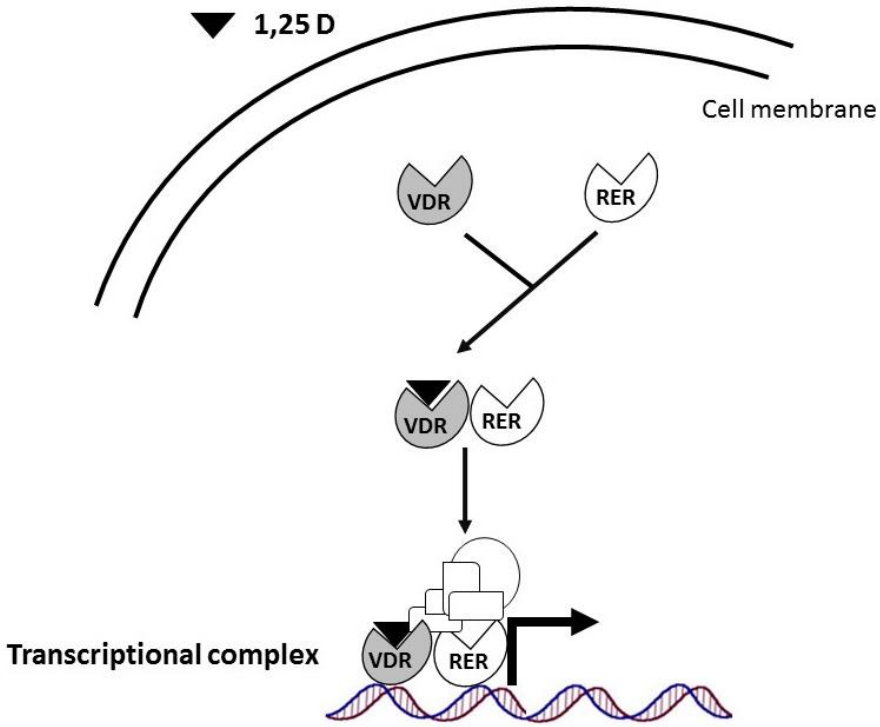


Figure 2.8 Vitamin D signaling pathways (90)

5.3 Potential effects of vitamin D in skeletal muscle

1,25$(OH)_2$D-VDR-RXR complex affects the regulation of a diversity of cellular functions such as DNA repair, cell differentiation and proliferation, apoptosis, and inflammation. Therefore, the role of vitamin D on muscle function seems to facilitate in a wide number of ways.

5.3.1 Muscle contraction and function

1,25$(OH)_2$D regulates muscle cell calcium influx by modulating the activity of calcium pumps and a calcium binding protein such as calbindin D9K, located in the sarcoplasmic reticulum and sarcolemma that regulate muscle contraction (5).

5.3.2 Muscle differentiation

1,25$(OH)_2$D motivates muscle cell differentiation through the regulation of several growth factors and inhibitors. Myostatin is a hormone produced and released by myocytes that acts to inhibit myogenesis (92). The previous study reported that vitamin D suppressed myostatin production from muscle cells. Garcia et al. demonstrated that the addition of 1,25$(OH)_2D_3$ to C_2C_{12} myoblasts increased myogenic

differentiation by raising the expression of insulin-like growth factor II (IGF-II) and follistatin and reducing the expression of myostatin (93). Moreover, the treatment of C_2C_{12} muscle cells with $1,25(OH)_2D_3$ enhanced vascular endothelial growth factor alpha (VEGFa) and fibroblast growth factor-1 (FGF-1) which both factors involve angiogenesis, tissue healing and muscle myogenesis. Whereas $1,25(OH)_2D_3$ decreased FGF-2 and tissue inhibitors of metalloproteinase-3 (TIMP-3) that promote angiogenic inhibitors (94).

5.3.3 Muscle inflammation

Vitamin D regulated the production of various pro-inflammatory cytokines such as interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α). In animal model, vitamin D_3 supplement 1000 IU/d for 8 weeks in rats with high-intensity exercise demonstrated a decrease in plasma levels of creatine kinase (CK) and gene expression of p38, ERK1/2, IKK, IκB, IL-6 and TNF-α as compared with sedentary controls. Moreover, in skeletal muscle of vitamin D treatment group had highly VDR protein expression (95).

5.4 Potential effects of vitamin D in bone

$1,25(OH)_2D$ is recognized by its receptor in osteoblasts and induces the expression of the receptor activator of nuclear factor-κB ligand (RANKL). RANKL binds RANK which is a receptor for RANKL on preosteoclasts leading to osteoclasts maturation. Mature osteoclasts remove calcium and phosphorus from bone, maintaining calcium and phosphorus levels in the blood (86). Moreover, $1,25(OH)_2D$ induced osteoblastic bone mineralization through VDR for the studies in OA osteoblasts have been shown an increased OA osteoblasts proliferation, increased bone formation and angiogenesis after vitamin D treatment. In OA chondrocytes, $1,25(OH)_2D$ involved an increased metalloproteinase (MMP) leading to cartilage degradation and osteoarthritic chondrocytes hypertrophy (37).

5.5 Classifications of vitamin D levels

The endocrine society recommends the optimal vitamin D level at 30 ng/ml to 100 ng/ml of circulating serum 25(OH)D level while vitamin D deficiency is defined as a 25(OH)D less than 20 ng/ml and vitamin D insufficiency is between 21 to 29 ng/ml as show in **Table 2.4** (96).

Table 2.4 Classification of vitamin D levels (96).

	25 (OH)D concentration	
Classification	**ng/ml**	**nmol/L**
Deficiency	< 20	< 50
Insufficiency	20-<30	50-<72.90
Sufficiency	30-100	72.90 - 250
Toxicity	> 150	> 375

5.6 Risk factors for vitamin D deficiency (86, 97)

There are several causes of vitamin D deficiency including:

5.6.1 Decreased skin synthesis

- Using sunscreen: decreased absorption of UVB radiation
- Skin pigment (Dark skin): decreased absorption of UVB radiation by melanin
- Aging (age older than 65 years): reduction of 7-dehydrocholesterol in the skin
- Season, latitude, and time of day

5.6.2 Decreased bioavailability

- Obesity (body mass index > 30 kg/m^2)
- Sedentary lifestyle

5.6.3 Medication use (such as anticonvulsants, glucocorticoids): increased vitamin D catabolism by destroying 25(OH)D and 1,25$(OH)_2$D to inactive calcitroic acid

5.6.4 Breastfed exclusively without vitamin D supplementation: low vitamin D

content in human milk results in increased vitamin D deficiency in infant

5.6.5 Reduced synthesis of 25(OH)D: Liver dysfunction

5.6.6 Reduced synthesis of $1,25(OH)_2D$: Chronic kidney disease

5.7 Vitamin D deficiency in OA

OA co-exists frequently with vitamin D deficiency in older people, Heidari et al. found that there was a high prevalence rate of vitamin D deficiency in patients with knee OA aged less than 60 years. Therefore, vitamin D deficiency was associated with initiation of early OA symptoms and the development of knee cartilage damage (14). Sanghi et al. found that the patients with primary knee OA had 63% vitamin D insufficient (25(OH)D $\leq$ 30 ng/ml) (13). In a Mediterranean country, the majority of patients with knee or hip OA scheduled for joint replacement were 81.7 % vitamin D deficiency; 15.2 % vitamin D insufficiency and only 3 % vitamin D sufficiency (98).

Pain is a major symptom of OA and limits physical activity that leads to decreasing their quality of life. A longitudinal study found that moderate vitamin D deficiency (25(OH) D = 12.5-25 nmo/l) could predict changes in knee pain over 5 years by using pain scores of the Western Ontario and McMaster University Osteoarthritis Index (WOMAC). In addition, the prevalence of moderate vitamin D deficiency was 4.2% and mild deficiency (25(OH) D= 25–49.9 nmo/l) was 37% (99). Recently, Kim et al. demonstrated that knee OA with vitamin D deficiency had poor health-related quality of life than these of normal vitamin D levels (100). However, currently studies have shown conflicting results in terms of the association between vitamin D and pain in OA. 25(OH)D levels was not significantly associated with severity of knee pain in elderly (101).

In addition, vitamin D deficiency was associated with radiographic knee OA. Bergink et al. reported that participants with low dietary vitamin D intake enhanced the risk of progressive development of radiographic knee OA (ROA). Moreover, the patients were not only low bone mineral density (BMD) but also low serum 25(OH)D levels (102). A recent study of the progression of knee OA found that patients with both low vitamin D status and high PTH had a high odds ratio (OR) of knee OA

progression (OR:3.2) by using joint space narrowing score (JSN) (103). Therefore, this study suggested that the participants with vitamin D deficiency have an increased risk of progressive knee OA.

Furthermore, low vitamin D levels affect muscle function. Barker et al. reported that knee OA with vitamin D deficiency had knee extensor isokinetic peak torques impairment (16). Similarly, the result of previous study showed a positive association between 25(OH)D levels and quadriceps muscle strength (104). Particularly, obesity individuals with low vitamin D status had poor physical performance than obese participants with normal vitamin D levels by using short physical performance battery test (105).

However, the conflicting results have been presented. Low vitamin D status was not related with incidence of OA and functional performance. A 22-year follow-up study of the incidence of knee and hip OA found that there was no association between 25(OH)D and the incidence of developing knee or hip OA (106). Al-Jarallah reported that high prevalence of vitamin D deficiency in Kuwait was 92.9% because of this cultural practice such as long clothing; otherwise there was no association between 25(OH)D levels, and functional assessment (107). Furthermore, low serum levels of 25(OH)D could not predict the outcomes of knee and hip OA undergoing hospitalization (108).

6. Vitamin D supplementation

It has been recognized that vitamin D is related with protein synthesis and the cell growth in skeletal muscles. Vitamin D supplementation may modify treatment in the elderly who are at risk of vitamin D deficiency and poor physical function or sarcopenia.

6.1 Vitamin D therapeutic uses

There are several types of vitamin D therapy and various forms of vitamin D exist. Terminology of vitamin D can indicate the following: ergocalciferol (vitamin D_2) and cholecalciferol (Vitamin D_3) are referred to as nutritional vitamin D. Calcidiol (25 hydroxyvitamin D), calcitriol (1,25-dihydroxyvitamin D), and alfacalcidol (1α-

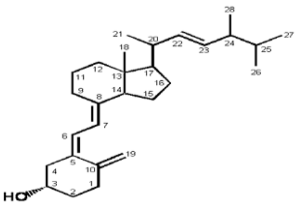
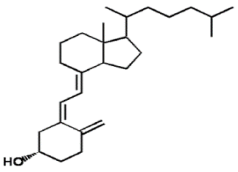
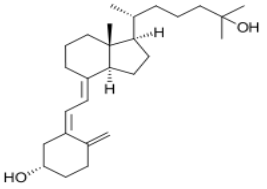
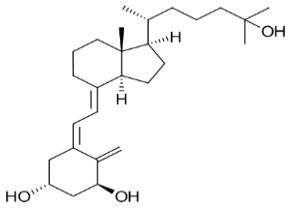
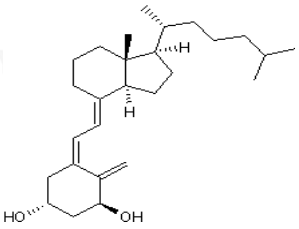
hydroxyvitamin D) are referred to as active vitamin D. The structure of vitamin D compound is similar to steroids (109) as shown in **Table 2.5**.

Vitamin D_2 is produced from ergosterol through UV irradiation in phytoplankton, yeast and mushrooms. Ergosterol is the principal sterol which enhanced with vitamin D_2. Whereas, vitamin D_3 is synthesized from 7-dehydrocholesterol through UV irradiation in skin and obtained from fortify milk, egg yolk and fatty fish (110). The differences of two chemicals are vitamin D_2 consist of 28 carbons and present a double bond between carbons 22 and 23 and a methyl group on carbon 24, whereas vitamin D_3 contains 27-carbon molecule (111). Both cholecalciferol and ergocalciferol are used in over-the-counter vitamin D treatments. The National List of Essential Medicines in Thailand identify ergocalciferol is a first-line drug (category A) for vitamin D deficiency that used in the hospitals and public health services in Thailand (112).

Vitamin D_2 and vitamin D_3 are metabolized in the liver to 25(OH)D and added a second OH group in kidney, while active vitamin D, particularly alfacalcidol is chemically synthesized by cholesterol and undergoes 25-hydroxylation in the liver to become fully activated into calcitriol. Only calcitriol does not need any hydroxylases and can bind VDR for exciting vitamin D related biological effects as shown in **Figure 2.9**. Therefore, active vitamin D is employed for therapeutic in osteoporosis, hypoparathyroidism, and kidney failure with impaired 1α-hydroxylase enzyme (113).

American Geriatrics Society Workgroup on vitamin D supplementation for older adults recommends an average daily input from all sources of 4,000 IU/d and the proposed upper tolerable level of 10,000 IU/d (88). The endocrine society suggests that the adults who are vitamin D deficiency treated with 50,000 IU of vitamin D_2 or vitamin D_3 once a week for 8 weeks or 6,000 IU/d of vitamin D_2 or vitamin D_3 and followed by maintenance therapy of 1500–2000 IU/d. To prevent recurrence of vitamin D deficiency, 50,000 IU of vitamin D_2 once every other week was effective to maintain levels of 25(OH)D (35-50 ng/ml) without any untoward toxicity (96). Sansanayudh et al. found that the patients received ergocalciferol 40,000 IU/week treatment for 8 weeks achieved a normal serum 25(OH)D level than 20,000 IU/week. For obese adults need at least two to three times more vitamin D (at least 6,000–10,000 IU/d) to treat and prevent vitamin D deficiency (114).

Table 2.5 The structure of vitamin D (109, 115, 116)

Vitamin D Form	Source	Structure
Ergocalciferol (Vitamin D_2)	Derived from plant sources or dietary supplements	
Cholecalciferol (Vitamin D_3)	Produced in human skin or derived from animal sources or dietary supplements	
Calcidiol (25 hydroxyvitamin D)	Produced in liver from cholecalciferol and ergocalciferol	
Calcitriol (1,25-dihydroxyvitamin D)	Produced in renal and extrarenal tissues or available in synthetic forms	
Alfacalcidol (1α-hydroxyvitamin D)	Synthetically produced	

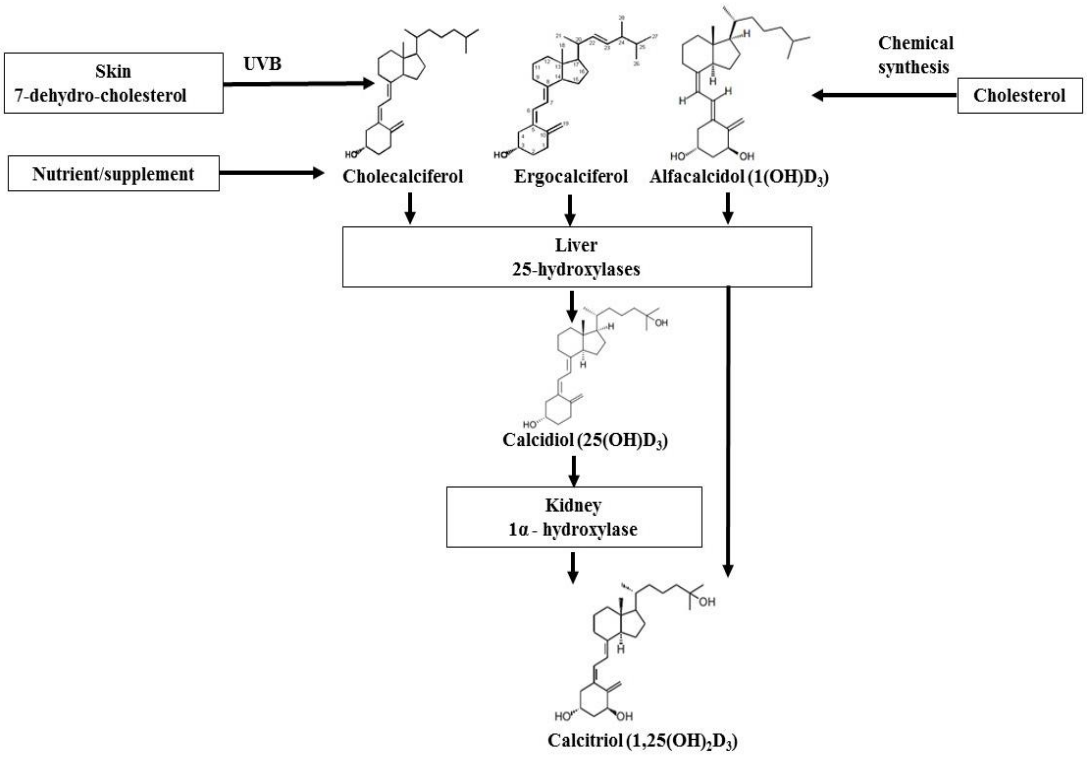


Figure 2.9 Vitamin D metabolism and drugs for clinical therapeutic uses (113).

6.2 Vitamin D supplementation, muscle strength, and physical performance

Several studies have reported that vitamin D supplementation increases muscle strength, improves physical function, and decrease the risk of falls in older females with low level of vitamin D. Bischoff et al. studied the effects of vitamin D and calcium supplementation on falls in elderly women. The subjects received 1,200 mg calcium plus 800 IU cholecalciferol (Cal + D-group) and 1,200 mg calcium alone (Cal-group) per day over 12-weeks. They found that musculoskeletal function such as lower limb strength, grip strength, and timed up & go test improved significantly in the Cal + D-group compared with the Cal-group. In addition, falls were reduced 49% in frail elderly women with vitamin D deficiency (17). Pfeifer et al. investigated the long-term effects of calcium and vitamin D on falls and parameters of muscle function in community-dwelling elderly women and men. The elderly has 25 (OH)D levels below 78 nmol/l received 1,000 mg of calcium (Cal-group) and 1,000 mg of calcium plus 800 IU of vitamin D (Cal + D-group) per day over a treatment period of 12 months. Cal +

D-group had significant improvements in quadriceps strength of 8%, a decrease in body sway of 28%, and a decrease in time of timed up & go test of 11% (19). A meta-analysis has shown that vitamin D supplementation alone is insufficient for decreased fracture risk. Vitamin D supplement of 800 IU per day with calcium may decrease the incidence of non-vertebral fractures, especially in the elderly having low-baseline vitamin D status and low calcium intake (117).

For the effect of vitamin D on skeletal muscle and function, Sato et al. found that treatment of poststroke hemiplegia in elderly with 1000 IU of vitamin D_2 daily significantly increased mean type II muscle fiber diameter and percentage of type II fibers over a 2 year period (20). Ceglia et al. reported the mobility-limited elderly women taking an oral vitamin D_3 4,000 IU/day or matching placebo for 4 months. They found that intramyonuclear VDR protein concentration was increasing 30% and total (type I and II) muscle fiber size was increasing 10% in 4-month period (21). For physical performance outcomes, the elderly were supplemented with 400 or 2,000 IU vitamin D_3 daily for 6 months. The elderly with the slowest gait speed improved their ability to perform chair-stand tests after vitamin D supplementation and higher supplemental doses were needed in persons who were overweight or obese (118).

However, some studies have reported that vitamin D supplementation did not improve muscle strength or physical function. Kenny et al. studied the effects of vitamin D supplementation in healthier populations of men. They took an oral vitamin D_3 1,000 IU/day and 500 mg calcium or matching placebo received 500 mg calcium for 4 months. They found that vitamin D supplementation (cholecalciferol) did not improve muscle strength or physical performance in a group of healthy community-dwelling older men. This study suggested that the effects of vitamin D supplementation should focus on individuals with low levels of vitamin D or impaired physical performance (22). Janssen et al. reported that vitamin D 400 IU/day plus 500 mg calcium supplementation did not improve muscle strength or mobility in vitamin D insufficient elderly females with various comorbidities (119). Oosterwerff et al. reported that moderate dose vitamin D_3 supplementation (1,200 IU/d) plus 500 mg calcium daily for 4 months did not have significant effects in 6 min walk test (6MWT) and physical performance scores in overweight and non-western immigrants (23).

6.3 Vitamin D supplementation in knee OA

For the effect of vitamin D on OA patients, McAlindon et al. studied knee OA patients received oral cholecalciferol 2,000 IU/d or placebo in 2 years. They suggested that vitamin D supplementation at a dose sufficient to elevate 25 (OH)D levels to more than 36 ng/mL did not reduce knee pain or cartilage volume loss and improve physical function (24). On the other hand, Sanghi et al. studied in knee OA patients with vitamin D insufficiency (25(OH)D < 50 nmol/L). Participants received oral vitamin D (cholecalciferol granules) of 60,000 IU per day for 10 days followed by 60,000 IU once a month or placebo for 12 months. At 12 months, the vitamin D group had decreased knee pain and improved knee function by using WOMAC and VAS assessment (13). More recently, Jin et al. demonstrated that symptomatic knee OA patients with low vitamin D status received a monthly capsule vitamin D_3 50,000 IU (1.25 mg) for 2 years. No reduction in tibial cartilage volume loss or improving WOMAC knee pain was observed (120) Arden et al. performed a 3 year RCT of 800 IU/d cholecalciferol in knee OA patients. The primary outcome was JSN and Secondary outcomes were WOMAC and the get up and go test. The study showed that vitamin D supplementation did not prevent radiological progression of knee joint or reduced joint pain, stiffness or improved physical performance (121).

According to all mentioned above, these findings suggest that vitamin D supplementation did not reduce knee pain or cartilage volume loss. Therefore, the role of vitamin D supplementation in knee OA patients on physical performance and strength remains a mystery. Thus, this study aimed to determine the effects of vitamin D_2 supplementation on muscle strength and physical performance in knee OA patients with low vitamin D status.

CHAPTER III

Materials and methods

1. Research design

The design of this study can be classified as a cross-sectional design. This study was approved by the Institutional Review Board on Human Research of the Faculty of Medicine, Chulalongkorn University, Thailand (IRB No. 512/57). Written informed consent was obtained from the patients prior to their participation in the study.

1.1 Target population

Knee OA patients aged between 50-80 years old with mild to moderate knee OA.

1.2 Sample population

Mild to moderate knee OA patients in orthopedics out-patient clinic at King Chulalongkorn Memorial Hospital and fulfill the criteria.

1.3 Inclusion criteria:

1. Knee OA patients who meet the criteria of American College of Rheumatology at least three out of six of the following criteria:
   - Age between 50-80 years old
   - Morning stiffness lasting less than 30 minutes
   - Crepitus on motion
   - Bony tenderness
   - Bony enlargement
   - No palpable warmth
2. Knee OA patients have vitamin D insufficiency (25(OH)D $\leq$ 30 ng/ml)
3. Individuals capable of giving informed consent on their own behalf
4. Individuals willing to attend in this study

1.4 Exclusion criteria:

1. Knee OA patients who have history of knee surgery
2. Known primary hyperparathyroidism
3. Current supplement of vitamin D
4. Kidney disease
5. Liver disease
6. Rheumatoid or other inflammatory arthritis (i.e. septic arthritis, gout).
7. Neurological condition (i.e. Parkinson's disease, previous stroke)
8. Severe cardiovascular condition (i.e. unstable angina)
9. Amputation of lower limbs
10. Unable to perform physical performance
11. Severe visual or hearing impairment uncorrected

1.5 Sample size

The sample size was calculated from Janssen et al., 2010 (122) studied the effects of vitamin D supplementation in females with vitamin D Insufficiency. Seventy females geriatric patients received vitamin D (cholecalciferol) 400 IU/day + calcium 500 mg/day (n=36) or placebo (placebo tablets + calcium 500 mg/day) (n=34) for 6 months. The supplementation group showed the mean serum 25 (OH)D level was significantly increased from 32.6±11.6 nmol/l to 77.2±19.4 nmol/l and the mean timed get up and go was 14.3±8.2 s to 13.1±7 s

$$n = (Z_{\alpha/2} + Z\beta)^2 \sigma^2/d^2$$

$\alpha = 0.05$ $Z_{\alpha/2} = Z_{0.05/2} = 1.96$ (two tail)

$Z\beta = 1.28$

$\sigma^2 = \sigma_1^2 + \sigma_2^2 - 2r\sigma_1 + \sigma_2$

$\sigma^2 = 1.44$

$d = 0.5$

$n = (1.96+1.28)^2 1.44/(0.5)^2$

$n = 61$

In order to compensation of subjects drop out or lost follow up, 100 knee OA patients were enrolled in this study.

1.6 Interventions

All participants with low vitamin D status were received two capsules of 20,000 IU ergocalciferol per week for 6 months (40,000 IU/week).

2. Sample collection

Data were collected during December 2015 to February 2016. Vitamin D status, biochemical markers, muscle mass, muscle strength and physical performance were assessed in knee OA patients as shown in **Figure 3.1**.

Knee OA patients with low vitamin D status received 40,000 IU ergocalciferol per week for 6 months. Serum 25 (OH)D, biochemical markers, muscle mass, muscle strength and physical performance were monitored at baseline and after ergocalciferol supplementation for 6 months as shown in **Figure 3.2**.

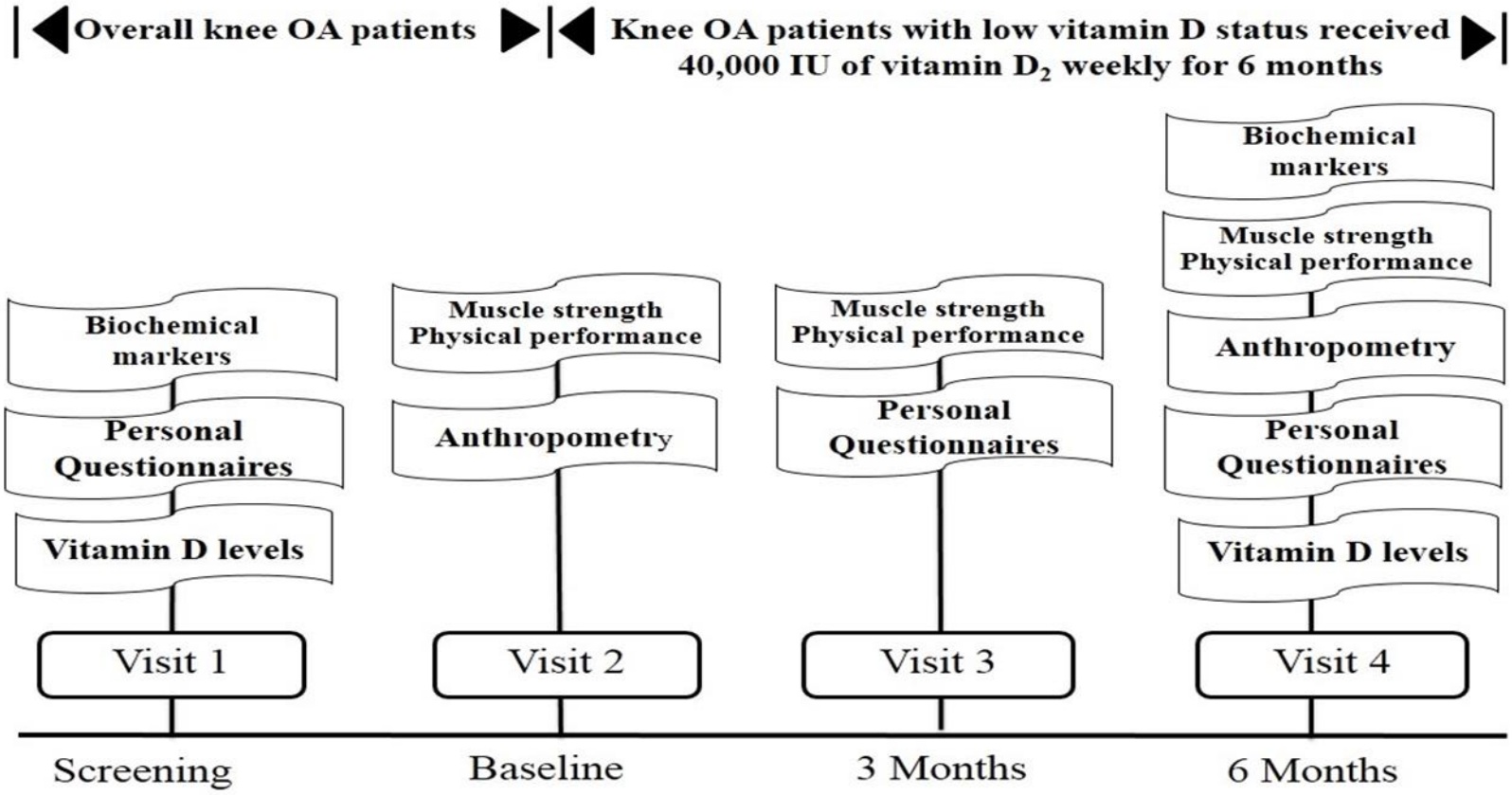


Figure 3.1 The study procedure in knee OA patients

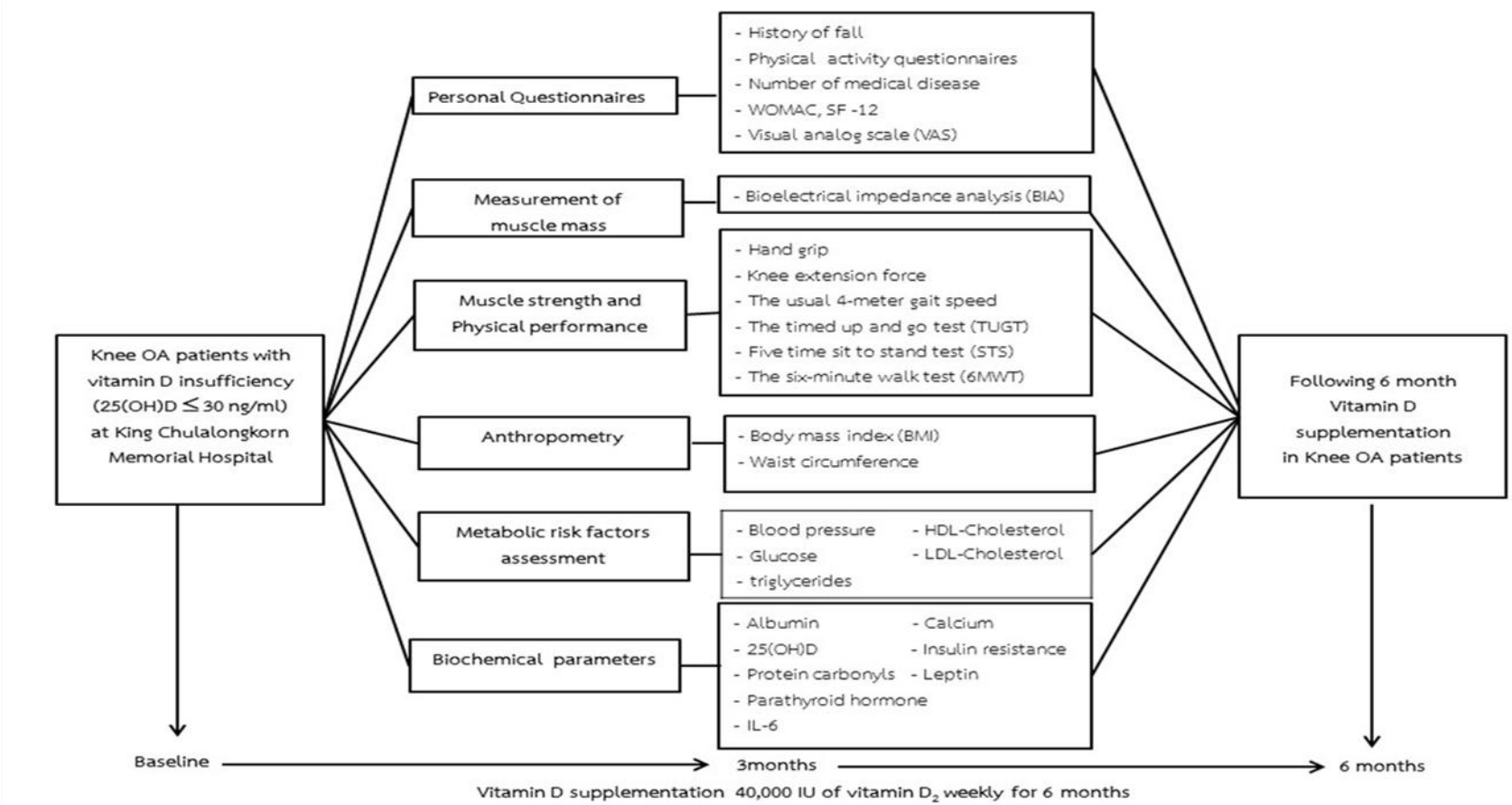


Figure 3.2 Physical examination and biochemical parameters in knee OA patients measured at baseline, 3 months and 6 months after vitamin D supplementation

3. Materials, equipment and reagents

3.1 Materials

- Aluminum foil (Rainbow metal company, USA)
- Clotted blood, EDTA and NaF tube (Vacuette, Austria)
- Disposable gloves
- Glass pipette: 5, 10 ml (Witeg, Germany)
- Microcentrifuge tube: 1.5 ml (Bio-Rad,USA)
- No 21, 23 needle, Sterile (Nipro,Thailand)
- Pipette tip: 10, 200, 1,000 µl (AxyGen,USA)
- Disposable syringe (Nipro, Thailand)
- 96-wells enzyme-linked immunosorbent assay (ELISA) plate
- Filter

3.2 Equipments

Equipments for laboratory

- Automatic adjustable micropipette (Eppendorf,Germany)
- Multichannel pipette
- Water bath (Memmert, German)
- Centrifuge 5804R (Eppendorf, USA)
- Vortex mixer (IKA Works, Malaysia)
- Spin drown centrifuge (Daihan scientific, Korea)
- Microplate reader (TECAN, USA)
- pH meter (Thermo Scientific, Germany)

Equipments for muscle mass

- Bioelectrical impedance analysis: BIA (Tanita BC-418, Japan)

Equipments for muscle strength

- Grip strength (Takei scientific instruments, Japan)
- Hand-held dynamometer (The microFET 2, USA)

Equipments for physical performance test

- Stop watch
- A standard height chair with 45cm seat height

4. Self-report assessment by questionnaire

4.1 Personal history questionnaire

- **History of fall**

The history of falls in the preceding 6 months prior to study enrollment was obtained by interviewing.

- **Number of medical disease**

The history of medical disease of participants in the study was assessed by interviewing.

4.2 Assessment of pain, stiffness, and physical disability: Western Ontario and MacMaster University (WOMAC) (123) and Visual analog scale (VAS)

Self-report pain, stiffness and physical disability were assessed by the Thai version of the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). The subscale consists of 24 items divided into 3 subscales: pain (5 items), stiffness (2 items) and physical function (17 items). The scale is displayed as ordinal scale of 0 – 10. A total WOMAC score is created by summing the items for all three subscales that a higher score on the WOMAC indicated worse pain, stiffness, and functional limitations.

4.3 12-Item short form health survey (SF-12) (124)

Thai version of the Short Form Health Survey (SF-12) assessed health-related quality of life including physical health composite scores (PCS), mental health composite scores (MCS) that range from 0 to 100 with higher points showed better self-reported health.

4.4 Physical activity questionnaire for elderly (125)

Physical activity was evaluated using the physical activity questionnaire for elderly Japanese (PAQ-EJ) in Thai version. The data were collected on the frequency (days/week) and duration (minutes/day). Participants self-reported the number of days and the time spent in the last seven days. The score (MET hr/week) = number of days ×time ×intensity weight.

5. Muscle mass

5.1 Measurement of muscle mass, fat mass and definitions of body composition categories.

Appendicular skeletal muscle mass (ASM) and fat mass were measured by bioelectrical impedance analysis (BIA: Tanita BC-418, Japan), for measuring impedances while patients standing on the metal foot-plates and holding hand grips for about 1 minute. The ASM can be measure by the sum of skeletal muscle mass of the arms and legs, except for the “trunk part”.

The skeletal muscle index (SMI) measures in terms of percentage of appendicular skeletal muscle mass (ASM) divided by body weight (%). SMI values lower than two standard deviations below the mean values of young male and female reference groups was classified as low muscle mass. Chang et al. (42) evaluated body composition by using bioelectric impedance analysis (BIA) with segmental measures in young adults. Mean SMI of young male adults are 36.30 $\pm$ 2.93 % and young female adults was 30.63 $\pm$ 2.41 %. Therefore, the cut-off low muscle mass or sarcopenia was SMI < 30.44 % in male and <25.81 % in female.

Figure 3.3 Body composition of participant was evaluated by using BIA

5.2 Anthropometry

5.2.1 Body mass index (BMI)

BMI was calculated as the weight (kg) divided by the square of the height (meters).

5.2.2 Waist circumference (WC)

WC was measured at the mid-point between the lower margin of the last palpable rib and the top of the iliac crest at the end of a normal expiration with the arms relaxed at the sides.

6. Muscle strength

6.1 Hand grip strength (126)

Hand grip strength was assessed by handgrip dynamometer. The best of three trials with the dominant hand was used for the present analysis. The cut-off used to evaluate strength reduction was < 26 kg for male and < 18 kg for female.

Figure 3.4 Grip strength was assessed by handgrip dynamometer

6.2 Knee extension force (127)

A maximum isometric knee extension force was measured by the microFET 2 hand-held dynamometer (HHD). The participant seated on the treatment table with lower legs perpendicular to the floor and knees flexed 90°. The dynamometer was applied to the anterior part of the leg, 5 cm above the transmalleolar axis and perpendicular to the tibial crest. The participant raised lower legs up 90°, parallel to the ground, and holed this position as strongly as possible against the maximum persistent (5s)

Figure 3.5 Knee extension force was measured by the microFET 2 hand-held dynamometer.

7. Physical performance

7.1 The usual 4-meter gait speed (126)

Participants were asked to walk 5 meters at a comfortable walking speed. The time to 4 meters walk was measured twice, excluding the starting and ending portions of 50 cm and the shorter time was selected. The cutoff value of gait speed to be used was 0.8 m/s.

Figure 3.6 Four meters gait speed was evaluated

7.2 Timed up and go test (TUGT) (128)

TUG test was used to assess the functional mobility. Time measurement required for taking to rise from a chair, walk three meters, turn around, walk back to the chair, and sit down. The cutoff value of TGUT to be used was 10 s.

Figure 3.7 Timed up and go test (TUGT) was performed

7.3 Sit to stand test (126)

For this test, participants were asked to rise from a standard height (45 cm) chair, five times as fast as possible with their arms folded. Participants undertook the test barefoot, and performance was measured in seconds, as the time from the initial seated position to the final seated position after completing five stands. The participant must complete the task in less than 12 seconds to pass the test.

Figure 3.8 Five time sit to stand test was evaluated

7.4 Six-minute walk test (6MWT) (128)

The participants were instructed to cover as much distance as possible at a self-paced walking velocity in a 25 meter flat surface within six minutes. They were allowed to rest during the test if necessary but was instructed to resume walking as soon as possible.

Figure 3.9 Six-minute walk test was evaluated

8. Blood pressure

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured after resting for at least 20 min in a sitting position using an auto- sphygmomanometer (Terumo Corporation, Japan).

9. Laboratory examination

9.1 Blood sampling and handling

Fasting blood samples were collected at both the baseline and the follow-up after vitamin D supplementation in 6 months. Peripheral venous blood samples of 10 ml were collected from each participant by standard venipuncture to sodium fluoride (NaF) tube, ethylene diamine tetraacetic acid (EDTA) tube and clotted blood tube. Subsequently, blood tubes were centrifuged at 4,000 rpm for 10 minutes at room temperature. Serum and plasma were separated and frozen at - 80°C until assayed and specimens were analyzed at Department of laboratory medicine at King Chulalongkorn Memorial Hospital.

9.2 Routine laboratory examination

Levels of fasting glucose, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), albumin, calcium, phosphate, high-sensitivity C-reactive protein (hs-CRP), Insulin, Parathyroid hormone (PTH), and 25(OH)D were determined by automatic spectrophotometric assay. Fasting glucose was determined by enzymatic (Hexokinase) method. Total cholesterol, HDL and triglycerides were assayed by enzymatic colorimetric assay. LDL, albumin, calcium and phosphate were determined by colorimetric assay and hs-CRP was measured using immunoturbidimetric method. PTH and insulin were determined electrochemiluminescent method (ECLIA). 25(OH)D and insulin were measured by chemiluminescent immunoassay (CLIA). Insulin resistance was calculated using Homeostasis Model Assessment (HOMA-IR).

HOMA-IR = fasting serum insulin (μU/ml) × fasting plasma glucose (mg/dL)

9.3 25(OH)D assay

25 (OH)D level was assay by LIAISON®25 (OH)D total assay (DiaSorin,USA) that used a direct competitive chemiluminescent immunoassay (CLIA) technology for the quantitative determination of 25-hydroxyvitamin D and other hydroxylated vitamin D metabolites in human serum on an automated platform.

Procedure

Frozen samples were put at room temperature to thaw and mix well before testing. The minimum volume of serum required was 250 µl for testing. The samples and calibrator were dispensed into the automated platform. Specific antibody against 25(OH)D was coated on magnetic particles (solid phase) and assay buffer were assigned into the automated platform. During the first incubation for 10 minutes, 25 (OH)D was dissociated from its binding protein and bound to the specific antibody on the solid phase. After 10 minutes, the tracer (25(OH)D conjugated to an isoluminol derivative) was added. After the second 10 min incubation, the unbound material was removed with a wash cycle. Finally, the starter reagents were added to initiate a flash chemiluminescent reaction. The light signal was measured by a photomultiplier as relative light units (RLU) and was inversely proportional to the concentration of 25(OH)D present in calibrators or samples.

9.4 Interleukin-6 (IL-6) assay

IL-6 levels were assayed by Human IL-6 ELISA MAX™ Standard Sets (BioLegend, USA).

Reagents

- Human IL-6 ELISA MAX™ Standard Sets (BioLegend, USA)
- Phosphate buffer saline (PBS)

  General reagents: Sodium chloride (NaCl), Potassium chloride (KCl), Sodium phosphate dibasic (Na_2HPO_4) and Potassium phosphate dibasic (KH_2PO_4)

  PBS Preparation:

  - Add 8 g of NaCl, 0.2 g of KCl, 0.204 g of KH_2PO_4, 1.44 g of Na_2HPO_4 to

1,000 ml with distilled water.

- Adjust to pH 7.4 and filter PBS by bottle top filter

- Coating buffer: 8.4 g $NaHCO_3$, 3.56 g Na_2CO_3, added DI H_2O to 1 L, pH to 9.5
- Tween®20 (Sigma-Aldrich, USA)
- Substrate solution: TMB substrate (BioLegend, USA)
- Stop solution: 2 N H_2SO_4 (Sigma-Aldrich, USA)

  Dilute 1.11 ml of H_2SO_4 to 18.99 ml with distilled water
- 10% Fetal bovine serum: FBS or Bovine serum albumin: BSA

Procedure

The capture antibody was diluted in coating buffer for 1:200 (for 1 plate, dilute 60 µl capture antibody in 11.94 ml coating buffer). After that 100 µl of the diluted capture antibody was added to coat a 96 well microplate and then incubated overnight at 4°C. Next day, reagents and samples were brought to room temperature. Each wells was aspirated and washed 4 times with 300 µl wash buffer (0.05% Tween®20 in PBS). Following this, 200 µl of assay diluent (10% FBS or 1% BSA in PBS) was added to block each wells, sealed microplate with an adhesive strip and incubated at room temperature with shacking at 200 rpm on a plate shaker for 1 hour. After second of four washes, 100 µl of serial standard diluent and sample was added to each well, sealed plate and incubated at room temperature for 2 hours with shaking. After third of four washes, the detection antibody was diluted in assay diluent for 1:200 (for 1 plate, dilute 60 µl detection antibody in 11.94 ml assay diluent). Following this, 100 µl of diluted detection antibody solution was added to each well, sealed and incubated at room temperature for 1 hours with shaking. After forth of four washes, the streptavidin-HRP was diluted in assay diluent for 1:1000. 100 µl of the dilution of streptavidin-HRP was added to each well, sealed and incubated at room temperature for 30 minutes with shaking. Final washing, each well was aspirated and washed 5 times with soaking for 30 seconds to 1 minute per wash. Following this, 100 µl of TMB substrate solution was added to each well and incubated in the dark for 15-30 minutes at room temperature. Finally, 100 µl of stop solution (2 N H_2SO_4) was added to each well. The concentration of IL-6 was determined within 30 minutes by using microplate reader. The optical density was recorded at 450 nm and 570 nm.

9.5 Leptin assay

Leptin concentration was assayed by enzyme-linked immunosorbent assay (ELISA) technique using the human leptin ELISA kit from R&D systems Inc., USA. DuoSet ELISA development kit contains the basic components required for the development of sandwich ELISAs to measure natural and recombinant human leptin.

Reagents

- DuoSet ELISA kit human leptin (R&D System, USA)
- Phosphate buffer saline (PBS)
- Tween®20 (Sigma-Aldrich, USA)
- Substrate solution: 3,3',5,5'-Tetramethylbenzidine: TMB (BioLegend, USA)
- Stop solution: 2 N H_2SO_4 (Sigma-Aldrich, USA)
- Bovine serum albumin: BSA
- Fetal bovine serum: FBS

Procedure

The capture antibody was diluted to the working concentration of 4 µg/ml in PBS without carrier protein. After that 100 µl of the diluted capture antibody was added to coat a 96 wells microplate and then incubated overnight at room temperature. Next day, frozen samples were put at room temperature to thaw completely for 30 minutes. The wells were aspirated and washed with 400 µl wash buffer (0.05% Tween®20 in PBS) for a total of 3 washes. 300 ml of reagent diluent (1% BSA and 10% FBS in PBS) was added to each well and incubated at room temperature for an hour. After second of three washes, 100 µl of sample (1: 50 reagent diluent) or standards diluted in reagent diluent were added to each well and incubated for 2 hours at room temperature. After third of three washes, the detection antibody was diluted to the working concentration of 25 ng/ml in reagent diluent (1% BSA in PBS). After that 100 µl of the detection antibody was added to each well and incubated for 2 hours at room temperature. After forth of three washes, 100 µl of substrate solution was added to each well and incubated for 20 minutes at room temperature that the wells avoided placing the plate in direct light. Finally, 50 µl of stop solution (2 N H_2SO_4)

was added to each well and gently taped the plate to ensure thorough mixing. Immediately, the concentration of leptin was recorded using microplate reader set to measurement wavelength at 450 nm and reference wavelength at 540 nm. The difference between measurement and reference measurement were calculated for the concentration of leptin by using standard curve.

9.6 Protein carbonyls assay

For the assessment of protein oxidation, protein carbonyl spectrophotometric assay utilizes the DNPH reaction to measure the protein carbonyl content in plasma.

Reagents

- Phosphate buffer saline (PBS)
- Dinitrophenylhydrazine: DNPH (TCI, Japan)
- Trichloroacetic acid: TCA (MP, Biomedicals, France)
- Ethanol (Merck, Germany)
- Bicinchoninic acid (BCA) Protein assay kit (Navagen®, USA)
- Ethyl acetate (Fisher Scientific, UK)
- Hydrochloric acid: HCL (NORMAPUR®, UK)
- Guanidine Hydrochloride: GdHCl (ACROS, Germany)

Reagent Preparation

20% TCA

- Add 20 g of TCA to 100 ml with distilled water.

2 N HCL

- Add 200 ml of HCL to 900 ml with distilled water

10 mM DNPH

- Dilute 0.991g of DNPH to 500 ml 2 N HCL

6 M GdHCl

- Add 34.023 g of potassium phosphate (KH_2PO_4) and 286.6g of GdHCl to 400 ml with distilled water.
- Adjust to pH 2.5 and add distilled water to total volume 500 ml

Procedure

Frozen plasma samples were brought to room temperature to thaw completely for least 20 minutes. Plasma sample was diluted in PBS for 1:20 (dilute 30 µl plasma in 570 µl PBS). 62.5 µl of diluted plasma was transferred to two 1.5 ml plastic tubes. One tube was the sample tube and the other was the control tube. 250 µl of 10mM DNPH was added to the sample tube and 250 µl of 2N HCL was added to control tube, incubated both tubes in the dark at room temperature for one hour and vortex each tube every 15 minutes during the incubation. After incubation, 300 µl of 20%TCA was added, vortex and incubated tubes on ice for 10 minutes. All tubes were centrifuged at 10,000 x g (rcf) for 10 minutes at 4°C in a microcentrifuge. The supernatant was discarded and wash the pellet 3 times with 625 µl of (1:1) Ethanol/Ethyl Acetate and centrifuge tubes at 10,000 x g (rcf) for 10 minutes at 4°C in a microcentrifuge. After the final wash, 300 µl of 6 M GdHCl was added and incubated at 60°C for 30 minutes. 250 µl of supernatant from sample and control tube was transferred to each well of the 96-well plate. Finally, the concentration of protein carbonyl (nmol/ml) was determined by using microplate reader. The absorbance was measured at 375 nm.

Calculation of results

The extinction coefficient (ε) for dinitrophenylhydrazine at 375 nm is 22,000 M^{-1} cm^{-1}. When the absorbance of the sample is read at 375 nm against its blank, the carbonyl content is calculated:

Protein Carbonyl (M) = OD_{375} / 22,000 M^{-1} (when 1 cm-width cuvette is used)

Protein Carbonyls (nmol/mL) = OD_{375} x 45.45 (nmol/mL)

Protein Carbonyls (nmol/mg) = $\frac{\text{Protein Carbonyls (nmol/mL)}}{\text{Protein Concentration (mg/mL)}}$

9.7 Protein concentration assay

The Bicinchoninic acid (BCA) protein assay is based on a biuret reaction, the reduction of Cu^{2+} to Cu^{1+} by protein in an alkaline solution, and a concentration-dependent detection of the monovalent copper ions (Cu^{1+}) produced.

Procedure

Plasma sample was diluted in PBS for 1:200 (dilute 1 µl plasma in 199 µl PBS). Subsequently, BCA working reagent was prepared by mixing 50 parts BCA solution with 1 part of 4% Cupric Sulfate (for each sample, 200 µl BCA solution and 4 µl 4% Cupric Sulfate). 25 µl of serial standard diluent and plasma diluent was added into wells of a 96 well microplate. Following this, 200 µl BCA working reagent was added to each well, mixed on plate shaker for 30 seconds, sealed and incubated at 37°C for 30 minutes. Finally, the concentration of protein sample (µg/ml) was determined by using microplate reader. The optical density was recorded at 562 nm.

10. Statistical analysis

Statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS software), v. 22.0 for Windows. The demographic data of knee OA patients between vitamin D deficiency, insufficiency and sufficiency group were analyzed by One way ANOVA and chi-squared test. Descriptive statistics were reported as mean ± standard error of mean (SEM). Spearman's correlation coefficient was used to calculate the association between leptin levels, body compositions, muscle strength and physical performance. Multiple regression analysis was performed to determine the association between serum vitamin D, leptin, hs-CRP, IL-6, protein carbonyls, muscle mass, muscle strength and physical performance.

The study of vitamin D supplementation, difference baseline and after vitamin D supplementation was determined by paired *t*-test. One-way repeated-measurement ANOVA was used to test the time differences in muscle strength and physical performance.

A *p*-value less than 0.05 was considered to be statistically significant for differences and correlations.

CHAPTER IV

Results

Participants

Two hundreds thirty-eight patients with knee OA enrolled in the study. The vitamin D status and prevalence of sarcopenia in knee OA patients were analzed. Then patients with vitamin D insufficiency and deficiency were asked to take 40,000 IU vitamin D_2 supplementation per week for 6 months. One hundred nighty-one met the criteria and was recruited in the study. Sixteen participants dropped out, 13 participants loss follow up, one had hip fracture, one had lower leg fracture and one had knee arthroscopy. Finally, a total of 175 participants (158 females and 17 males) were completely received vitamin D_2 supplementation for 6 months during December 2014 to February 1016 as shown in **Figure 4.1**.

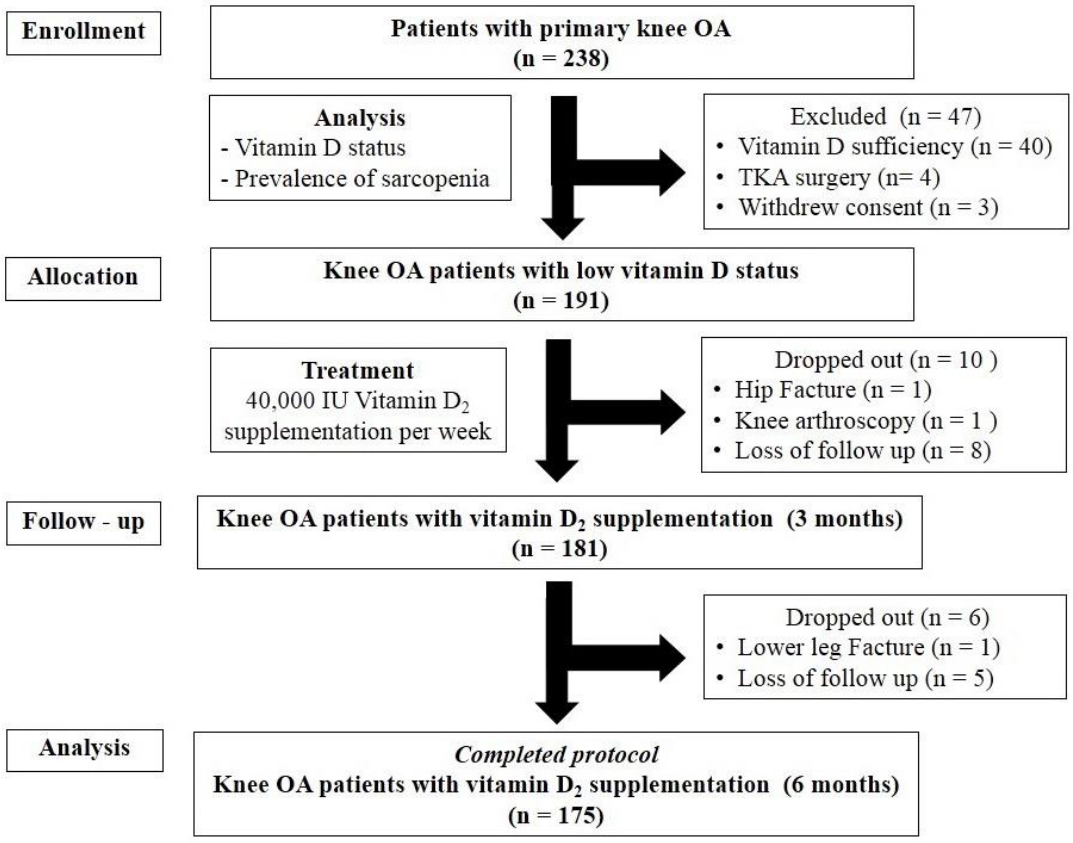


Figure 4.1 Flowchart of participants in the study

Baseline characteristics of knee OA participants

A total of 238 knee OA patients were enrolled in the study. Mean age of participants was 65.35±0.46 years and 90.33 % of participants were females and mean of 25(OH)D levels was 23.52±0.54 ng/ml.

After vitamin D_2 supplementation for 6 months during December 2014 to February 1016, 175 participants (158 females and 17 males) were completely supplemented. The mean age was 64.58±0.45 years and mean of 25(OH)D levels was 20.73±0.36 ng/ml as presented in **Table 4.1**.

Table 4.1 Baseline characteristics of knee OA participants

	Enrollment (n=238)	**Vitamin D supplementation (n=175)**
Characteristics	**Mean±SEM**	**Mean±SEM**
Age (years)	65.35±0.46	64.58±0.45
Gender (F/M)	215:23	158:17
Body composition		
BMI (kg/m^2)	25.46±0.26	25.63±0.30
Waist circumference (cm)	87.69±0.64	87.84±0.73
Percent of fat (%)	35.36±0.47	35.42±0.52
Fat mass (kg)	22.58±0.53	22.66±0.59
Visceral fat rating	9.57±0.25	9.46±0.29
SMI (%)	28.46±0.24	28.37±0.27
VAS (0-10 cm)	4.05±0.16	3.96±0.17
WOMAC		
Pain (0-10)	2.59±0.13	2.45±0.15
Stiffness (0-10)	2.68±0.16	2.56±0.18
Physical disability (0-10)	3.02±0.14	2.90±0.15
Total score (0-10)	2.77±0.13	2.80±0.13
SF-12		
PCS (0-100)	38.27±0.58	38.26±0.65
MCS (0-100)	48.66±0.59	50.00±0.70
Physical activity		
PAQ-EJ (MET hours/week)	52.23±2.79	52.28±2.83
25(OH)D (ng/ml)	23.52±0.54	20.73±0.36

F: Female, M: Male, BMI: Body mass index, SMI: Skeletal muscle index, PCS: Physical health composite scores, MCS: Mental health composite scores, PAQ-EJ: physical activity questionnaire for elderly Japanese in Thai version, and 25(OH)D: 25-hydroxyvitamin D

Part I Comparative studies in knee OA patients according to vitamin D status (n=238)

All knee OA patients (n=238) were studied according to physical assessments and biochemical characteristics: the physical assessments included body composition, muscle strength, physical performance and quality of life. The biological studies were dealed with all the biomarkers involving the metabolic risk factors and MetS, vitamin D, calcium and phosphorus homeostasis, inflammation, adipokine and oxidative stress.

Serum 25(OH)D levels measured in 238 patients showed a mean vitamin D level was 23.52 ng/ml. In 40 patients (16.80%), vitamin D levels were sufficient with 25(OH)D > 30.00 ng/ml, 118 patients (49.60%) had vitamin D insufficiency and 80 patients (33.60%) had vitamin D deficiency. Therefore, vitamin D insufficiency were highly prevalent in knee OA patients as shown in **Figure 4.2**.

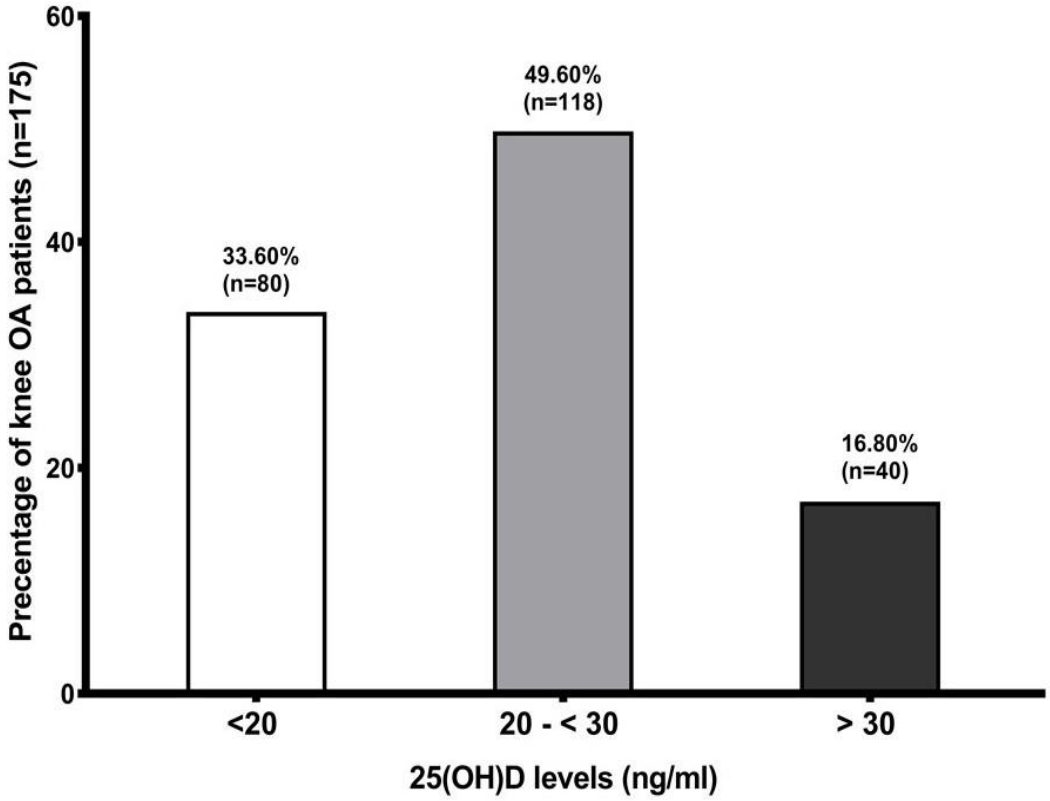


Figure 4.2 The prevalance of vitamin D insufficiency and deficiency in knee OA patients

1.1 Age and body composition in knee OA patients classified according to vitamin D levels

A total of 238 participants, 215 females and 23 males were categorized into 3 groups, vitamin D sufficiency, insufficiency and deficiency. The body composition data of three groups are shown in **Table 4.2**. Vitamin D deficiency was observed in younger participants (63.90±0.89 years) when compared to sufficiency groups (67.55±1.06 years, p = 0.02). Whereas, BMI, percentage of fat mass, fat mass, visceral fat rating and muscle mass were not different among three groups ($p > 0.05$).

Table 4.2 The demographic data of knee OA patients with vitamin D deficiency, insufficiency and sufficiency

	Vitamin D status			
	Sufficiency	**Insufficiency**	**Deficiency**	***p***
	n = 40	**n = 118**	**n = 80**	
		Mean ± SEM		
Gender (F/M)	35:5	105:13	75:5	
Age (years)	67.55±1.06	65.59±0.59	63.90±0.89 [a]	0.02
Body composition				
BMI (kg/m^2)	24.02±0.61	26.02±0.38	25.36±0.45	0.27
Percentage of fat mass (%)	33.01±1.25	36.04±0.64	35.50±0.79	0.07
Fat mass (kg)	20.07±1.27	23.46±0.74	22.52±0.91	0.08
Visceral fat rating	9.05±0.62	10.05±0.37	9.11±0.39	0.16
ASM (kg)	17.17±0.69	17.77±0.39	17.44±0.32	0.67
ASMI (kg/m^2)	6.96±0.19	7.27±0.12	7.14±0.09	0.34
SMI (%)	29.24±0.67	28.15±0.35	28.54±0.40	0.30

F: female, M: male, BMI: Body mass index, ASM: Appendicular skeletal muscle mass, ASMI: Appendicular skeletal muscle mass index, and SMI: Skeletal muscle index

[a] $p < 0.05$ deficiency versus sufficiency by analysis of variance with post hoc analysis

1.2 Muscle strength and physical performance in knee OA patients according to vitamin D levels

Knee OA patients with vitamin D deficiency had only significantly lower knee extension force both symptomatic (348.25±8.55 N) and non-symptomatic leg (365.99±9.11 N) than vitamin D insufficiency and sufficiency group ($p < 0.01$) but grip strength and physical performance were not different among three groups are shown in **Table 4.3.**

Table 4.3 Muscle strength and physical performance in knee OA patients according to vitamin D levels

	Vitamin D status			
	Sufficiency	**Insufficiency**	**Deficiency**	***p***
	n = 40	**n = 118**	**n = 80**	
	Mean ± SEM			
Muscle strength				
Grip strength:				
Dominant (kg)	22.62±1.05	22.78±0.51	21.25±0.53	0.14
Non dominant (kg)	21.19±1.08	20.62±0.49	19.19±0.56	0.10
Knee extension force:				
Symptomatic leg (N)	413.89±17.87	374.80±8.62	348.25±8.55 [a]	0.001
Non-symptomatic leg (N)	443.89±16.11	395.33±7.16	365.99±9.11 [b]	<0.001
Physical performances				
Gait speed (m/s)	1.00±0.03	0.94±0.01	0.96±0.02	0.23
TUGT (s)	9.44±0.36	10.09±0.22	9.75±0.32	0.34
STS (s)	14.19±0.73	15.22±0.42	14.99±0.53	0.48
6MWT (m)	391.52±13.34	366.25±7.11	366.03±9.81	0.20

TUGT: Timed up and go test, STS: sit to stand, and 6MWT: Six-Minute Walk test.

[a] $p < 0.05$ deficiency versus sufficiency by analysis of variance with post hoc analysis

[b] $p < 0.05$ deficiency versus insufficiency and sufficiency by analysis of variance with post hoc analysis

We further explored the prevalence of sarcopenia in knee OA patients using the Asian Working Group for Sarcopenia (AWGS) algorithm for sarcopenia. Sarcopenia was characterized by the presence of both low muscle mass with poor physical performance or muscle strength whereas pre-sarcopenia was defined as only low muscle mass. The prevalence of sarcopenia, pre-sarcopenia, and non-sarcopenia in knee OA patients were 10.10%, 12.60%, and 77.40%, respectively as shown in **Appendix A.**

The percentage number of knee OA patients who were sarcopenia, pre-sarcopenia and non-sarcopenia among vitamin D deficiency, insufficiency and sufficiency groups are presented in **Figure 4.3**. There was 12.50% (n=5) of sarcopenia in sufficiency group, 11.10% (n=13) and 7.40% (n=6) of sarcopenia in insufficiency and deficiency group respectively.

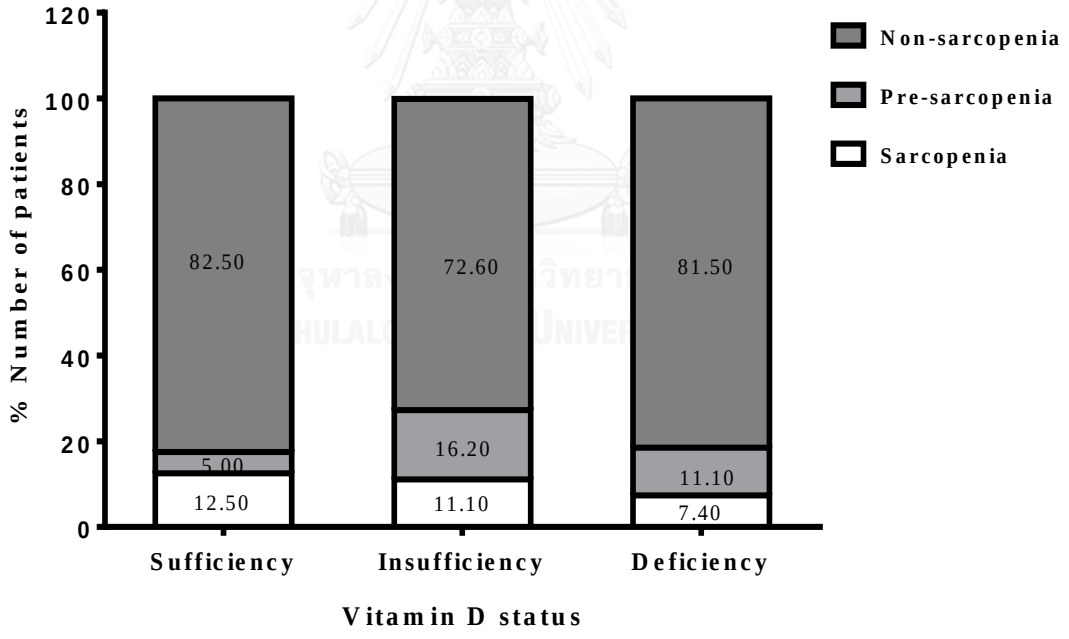


Figure 4.3 The percentage of knee OA patients with non-sarcopenia, pre-sarcopenia and sarcopenia among vitamin D deficiency, insufficiency and sufficiency groups.

1.3 Self-report pain, health-related quality of life and physical activity in knee OA patients with vitamin D deficiency

There was no difference in self-report pain, health-related quality of life and physical activity among vitamin D deficiency, insufficiency and sufficiency groups ($p > 0.05$) as shown in **Table 4.4**.

Table 4.4 VAS, WOMAC, SF-12 and PAQ-EJ in knee OA patients with vitamin D deficiency, insufficiency and sufficiency

	Vitamin D status			
	Sufficiency	**Insufficiency**	**Deficiency**	***p***
	n = 40	**n = 118**	**n = 80**	
		Mean ± SEM		
VAS (0-10)	3.82±0.42	4.16±0.22	4.00±0.27	0.75
WOMAC				
Pain (0-10)	2.45±0.32	2.55±0.19	2.72±0.24	0.77
Stiffness (0-10)	2.64±0.41	2.83±0.22	2.50±0.28	0.66
Physical disability (0-10)	2.93±0.36	3.02±0.19	3.07±0.24	0.95
Total score (0-10)	2.68±0.30	2.80±0.18	2.76±0.22	0.94
SF-12				
PCS (0-100)	40.28±1.52	37.85±0.85	37.95±0.95	0.33
MCS (0-100)	51.24±1.31	49.63±0.88	48.96±1.01	0.45
Physical activity				
PAQ-EJ (MET hours/week)	55.88±9.93	53.08±3.56	49.21±4.23	0.69

VAS: Visual analogue scale, WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index, SF-12: 12-Item short form health survey, PCS: Physical health composite scores, MCS: Mental health composite scores, PAQ-EJ: physical activity questionnaire for elderly Japanese in Thai version and MET: metabolic equivalent of task.

1.4 Frequency of falls in knee OA patients with vitamin D deficiency

Frequency of falls in knee OA patients during the past 6 months were observed as 37.50% in vitamin D deficiency group, 27.20% and 22.20% in vitamin D insufficiency and sufficiency groups respectively. There was no difference in fall rates among three groups ($p > 0.05$) as shown in **Table 4.5**.

The odds ratio (OR) of fall rates of vitamin D deficiency compared to vitamin D sufficiency groups was 0.62. However, there was no different in fall risk between groups ($p = 0.29$).

Table 4.5 Frequency of falls in the past 6 months in knee OA patients with vitamin D deficiency

	Vitamin D status			
	Sufficiency	Insufficiency	Deficiency	*p*
	n = 40	n = 118	n = 80	
	%			
Frequency of falls in 6 months				
No (%)	22.20	27.20	37.50	
Yes (%)	77.80	72.80	62.50	0.16

1.5 Metabolic risk factors and metabolic syndrome (MetS) in knee OA patients according to vitamin D levels

Knee OA patients with vitamin D deficiency had significantly higher levels of LDL cholesterol (141.27±4.51 mg/dl) and triglyceride (127.87±6.08 mg/dl) than those with vitamin D insufficiency and sufficiency groups ($p < 0.05$).

The prevalence rates of MetS were 32.50% in vitamin D sufficiency, 47.90% in vitamin D insufficiency and 40.70% in vitamin D deficiency group. These was no difference in MetS among three groups (p>0.05) as shown in **Table 4.6.**

Table 4.6 Metabolic risk factors and MetS in knee OA patients with vitamin D deficiency

	Vitamin D status			
	Sufficiency	**Insufficiency**	**Deficiency**	***p***
	n = 40	**n = 118**	**n = 80**	
		Mean ± SEM		
Metabolic risk factors				
Insulin (μU/ml)	5.03±0.67	5.71±0.50	5.21±0.57	0.69
HOMA-IR	1.36±0.21	1.46±0.14	1.31±0.18	0.81
LDL cholesterol (mg/dl)	120.58±4.60	133.55±3.00	141.27±4.51 [a]	0.01
MetS components				
Waist circumference (cm)	84.83±1.62	89.25±0.88	86.83±1.11	0.27
FBG (mg/dl)	102.05±3.24	98.64±1.62	98.85±2.83	0.66
HDL cholesterol (mg/dl)	59.87±2.69	53.51±1.22	56.18±1.36	0.49
Triglyceride (mg/dl)	95.64±6.11	122.26±4.90	127.87±6.08 [a]	<0.001
SBP (mmHg)	130.97±1.38	130.64±0.97	130.66±0.69	0.97
DBP (mmHg)	79.62±1.22	80.59±1.11	78.96±0.72	0.51
Number of component of MetS				
≤1 (%)	35.00	28.20	34.60	
2 (%)	32.50	23.90	24.70	
≥3: MetS (%)	32.50	47.90	40.70	0.48

HOMA-IR: Homeostatic model assessment of insulin resistance, FBG: Fasting blood glucose, SBP: Systolic blood pressure and DBP: Diastolic blood pressure

[a] $p < 0.05$ deficiency versus sufficiency by analysis of variance with post hoc analysis

1.6 Vitamin D, calcium and phosphorus homeostasis in knee OA patients according to vitamin D levels

Only knee OA patients with vitamin D deficiency had significantly lower levels of PTH (57.90±3.84 pg/ml) than those with vitamin D insufficiency (50.60±1.93 pg/ml) and sufficiency group (39.87±2.11 pg/ml, $p < 0.001$) as shown in **Table 4.7.**

Albumin, calcium and phosphorus were not different among three groups ($p > 0.05$).

Table 4.7 Vitamin D, calcium and phosphorus homeostasis in knee OA patients according to vitamin D levels

	Vitamin D status			
	Sufficiency	**Insufficiency**	**Deficiency**	***p***
	n = 40	**n = 118**	**n = 80**	
		Mean ± SEM		
25 (OH)D (ng/ml)	36.94±1.45	24.02±0.27	16.08±0.345 [a]	<0.001
PTH (pg/ml)	39.87±2.11	50.60±1.93	57.90±3.84 [a]	<0.001
Albumin (mg/dl)	4.32±0.03	4.27±0.01	4.30±0.02	0.34
Calcium (mg/dl)	9.16±0.20	9.26±0.03	9.24±0.05	0.69
Phosphorus (mg/dl)	3.66±0.06	3.62±0.03	3.62±0.05	0.84

25(OH)D: 25-hydroxyvitamin D and PTH: parathyroid hormone

[a] $p < 0.05$ deficiency versus insufficiency and sufficiency by analysis of variance with post hoc analysis

1.7 Inflammation, adipokine and oxidative stress in knee OA patients according to vitamin D levels

Levels of Il-6 (17.18±1.44 pg/ml), leptin (28.99±2.91 ng/ml) and protein carbonyls (0.650±0.06 nmol/mg) in knee OA patients with vitamin D deficiency were higher than vitamin D sufficiency group ($p < 0.001$) but hs-CRP had no difference among three groups ($p > 0.05$) (**Table 4.8).**

Table 4.8 Levels of inflammation (hs-CRP, IL-6), adipokine (leptin) and oxidative stress (protein carbonyl) according to vitamin D levels.

	Vitamin D status			
	Sufficiency	**Insufficiency**	**Deficiency**	***p***
	n = 40	**n = 118**	**n = 80**	
		Mean ± SEM		
hs-CRP (mg/dl)	1.32±0.27	2.22±0.32	1.81±0.19	0.19
IL-6 (pg/ml)	7.60±0.33	11.54±0.93	17.18±1.44 [a]	<0.001
Leptin (ng/ml)	17.53±2.16	26.51±2.04	28.99±2.91 [a]	0.03
Protein carbonyls (nmol/mg)	0.19±0.02	0.78±0.05	0.650±0.06 [a]	<0.001

hs-CRP: high-sensitivity C-reactive protein, IL-6: interleukin-6

[a] $p < 0.05$ deficiency versus sufficiency by analysis of variance with post hoc analysis

Part II Comparative studies in knee OA patients with vitamin D supplementation (n=175)

A total of 175 participants were completely treated with vitamin D_2 supplement for 6 months during December 2014 to February 2016. At baseline, the mean serum 25(OH)D levels in knee osteoarthritis patients was 20.73±0.36 ng/ml. Seventy-two participants (41.10%) were vitamin D deficient and 103 participants (58.90%) were vitamin D insufficient

After 40,000 IU of vitamin D_2 supplementation for 6 months, the mean serum 25(OH) D level was 32.14±0.59 ng/ml. One hundred knee OA participants (57.10%) were achieving serum 25(OH)D concentration above 30 ng/ml, 70 knee OA patients (40.00 %) had vitamin D insufficiency and only 5 patients (2.90%) had vitamin D deficiency after vitamin D_2 supplementation for 6 months **(Figure 4.4).**

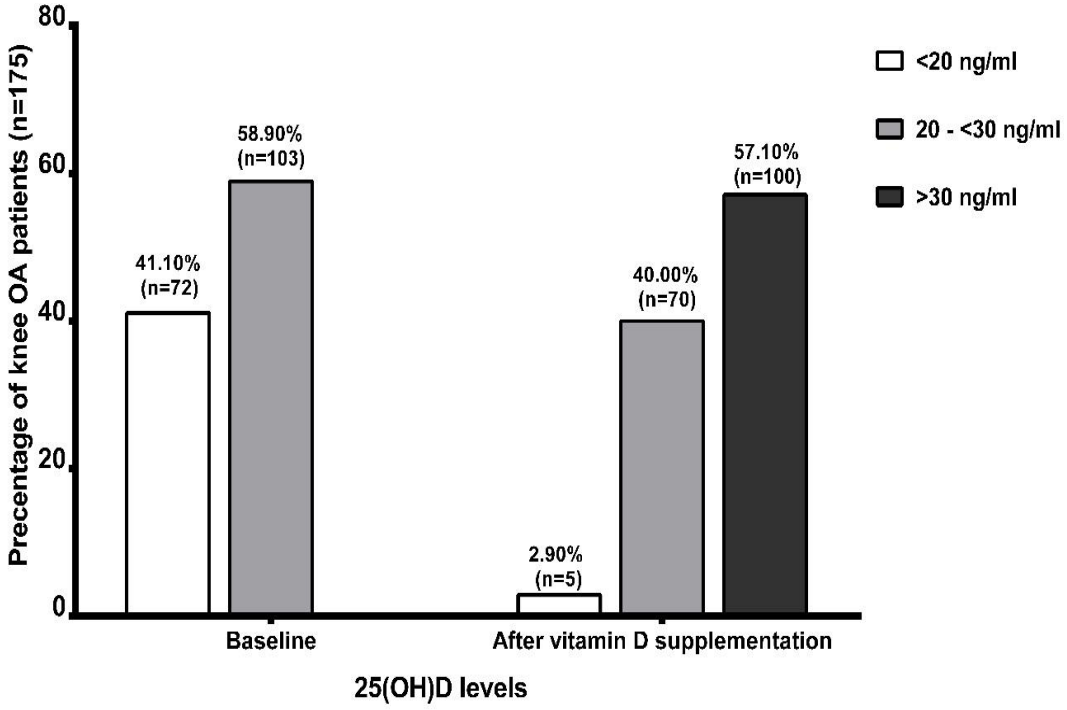


Figure 4.4 Vitamin D status in knee OA patients **A)** At baseline **B)** After 6 months vitamin D_2 supplementation

The percentage of knee OA patients who achieved optimal vitamin D level after vitamin D_2 supplementation was observed. In vitamin D deficiency group, 38.90% (n=28) knee OA patients achieved normal vitamin D levels, 54.20% (n=39) patients had vitamin D insufficiency and only 6.90% (n =5) patients remained vitamin D deficiency after vitamin D_2 supplementation. In addition, the results showed that 69.90% (n=72) knee OA patients who were in vitamin D insufficiency group at baseline had increased vitamin D level up to normal level. Only 30.10% (n=31) patients were remained classified as insufficiency after 6 months supplementation **(Figure 4.5)**.

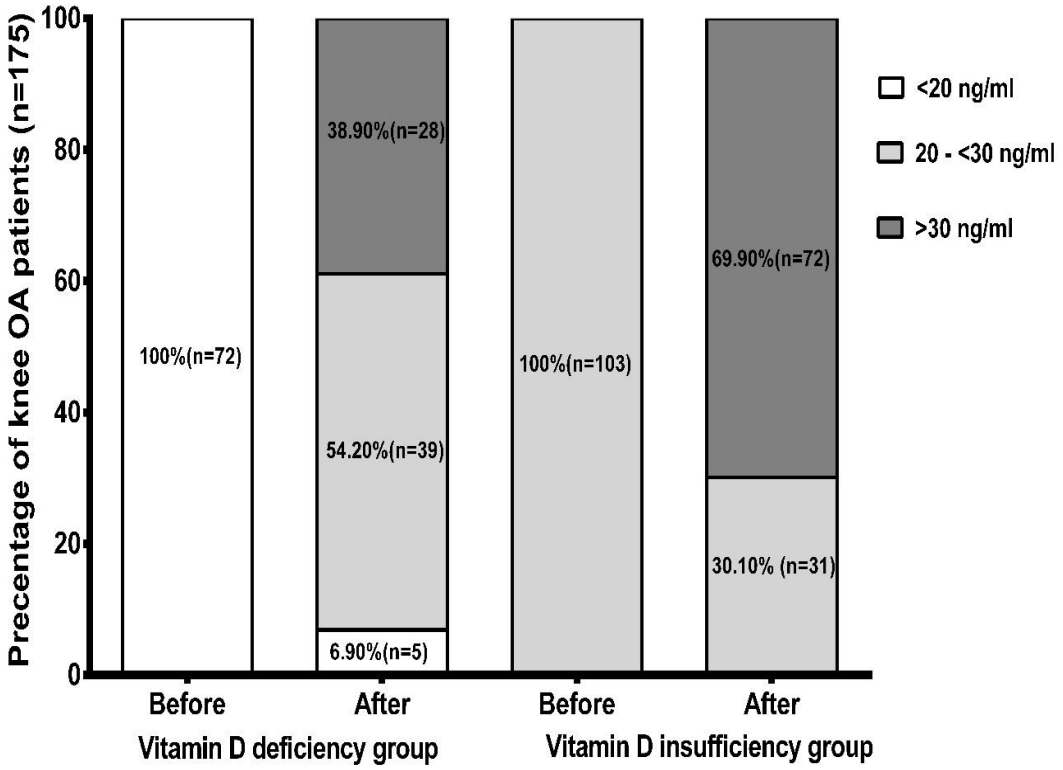


Figure 4.5 25(OH)D levels before and after vitamin D_2 supplementation between vitamin D deficiency and vitamin D insufficiency group at baseline.

2.1 Age and body composition in knee OA patients with vitamin D supplementation

One hundred seventy-five participants (158 females and 17 males) were completely treated with vitamin D_2 supplement for 6 months. The mean age was 64.58±0.55 years.

The differences between before and after vitamin D_2 supplementation were shown in **Table 4.9**. Body weight (62.38±0.89 vs 61.70±0.88 kg, p = 0.02), percentage of fat (35.42±0.52 vs 33.28±0.52 kg, $p < 0.001$), fat mass (22.66±0.59 vs 20.93±0.54 kg) and visceral fat rating (9.46±0.29 vs 9.03±0.24, p = 0.004) significantly decreased compared between before and after vitamin D supplementation. However, there was no significant difference change of muscle mass (SMI: 28.37±0.27 vs 28.52±0.25, $p > 0.05$).

Table 4.9 Age and body composition data before and after vitamin D_2 supplementation

	Vitamin D_2 supplementation (n=175)			
	Mean ± SEM		Mean Difference (95% CI)	*p*
	Before	After		
Gender (F/M)	158:17	158:17		
Age (years)	64.58±0.55	64.58±0.55		
Body composition				
Weight (kg)	62.38±0.89	61.70±0.88	-0.68 (-1.27to -0.09)	0.02
BMI (kg/m^2)	25.63±0.30	25.41±0.30	-0.22 (-0.48 to 0.04)	0.09
Percentage of fat (%)	35.42±0.52	33.28±0.52	-2.14 (-2.80 to -1.47)	<0.001
Fat mass (kg)	22.66±0.59	20.93±0.54	-1.72 (-2.30 to -1.14)	<0.001
Visceral fat rating (range)	9.46±0.29	9.03±0.24	-0.43 (-0.72 to -0.13)	0.004
ASM (kg)	17.58±0.28	17.50±0.27	-0.08 (-0.20 to 0.03)	0.15
ASMI (kg/m^2)	7.20±0.08	7.18±0.08	-0.03 (-0.08 to 0.01)	0.18
SMI (%)	28.37±0.27	28.52±0.25	0.14 (- 0.15 to 0.44)	0.33

F: female, M: male, BMI: Body mass index, ASM: Appendicular skeletal muscle mass, ASMI: Appendicular skeletal muscle mass index, and SMI: Skeletal muscle index

2.2 Effects of vitamin D_2 supplementation on muscle strength and physical performance

Significant improvement of muscle strength was observed in dominant grip strength (22.40±0.41 vs 23.05±0.41, p=0.006), gait speed (0.96±0.01 vs 1.14±0.01m/s, p <0.001), TUGT (9.81±0.19 vs 8.65±0.17 s, p <0.001), STS (14.87±0.36 vs 13.28±0.39 s, p <0.001) and 6MWT (371.22±5.95 vs 421.20±5.83 m, p <0.001) after vitamin D_2 supplementation as shown in **Table 4.10.**

However, the correlations between 25(OH)D levels, muscle strength and physical performance were not significant different at baseline or after treatment (p > 0.05).

Table 4.10 Muscle strength and physical performance after vitamin D_2 supplementation

	Vitamin D_2 supplementation (n=175)			
	Mean ± SEM		Mean Difference (95% CI)	*p*
	Before	After		
Muscle strength				
Grip strength:				
Dominant (kg)	22.40±0.41	23.05±0.41	0.65 (0.19 to 1.11)	0.006
Non dominant (kg)	20.26±0.40	20.44±0.40	0.18 (-0.28 to 0.64)	0.44
Knee extension force:				
Symptomatic leg (N)	356.01±5.95	358.61±5.38	2.59 (-4.42 to 9.62)	0.46
Non-symptomatic leg (N)	378.22±5.84	379.91±5.79	1.69 (-0.78 to 4.16)	0.18
Physical performances				
Gait speed (m/s)	0.96±0.01	1.14±0.01	0.18 (0.14 to 0.21)	<0.001
TUGT (s)	9.81±0.19	8.65±0.17	-1.16 (-1.48 to -0.83)	<0.001
STS (s)	14.87±0.36	13.28±0.39	-1.58 (-2.26 to -0.89)	<0.001
6MWT (m)	371.22±5.95	421.20±5.83	49.97 (40.46 to 59.48)	<0.001

TUGT: Timed up and go test, STS: sit to stand, and 6MWT: Six-Minute Walk test.

Muscle strength and physical performance at baseline, 3 and 6 months after vitamin D supplementation were further analysed. Knee OA patients had increased (A) grip strength of dominant side (p=0.01) and improved all physical performances such as (E) gait speed ($p < 0.001$), (F) TUGT ($p < 0.001$), (G) STS ($p < 0.001$), and (H) 6MWT ($p < 0.001$) as presented in **Figure 4.6.**

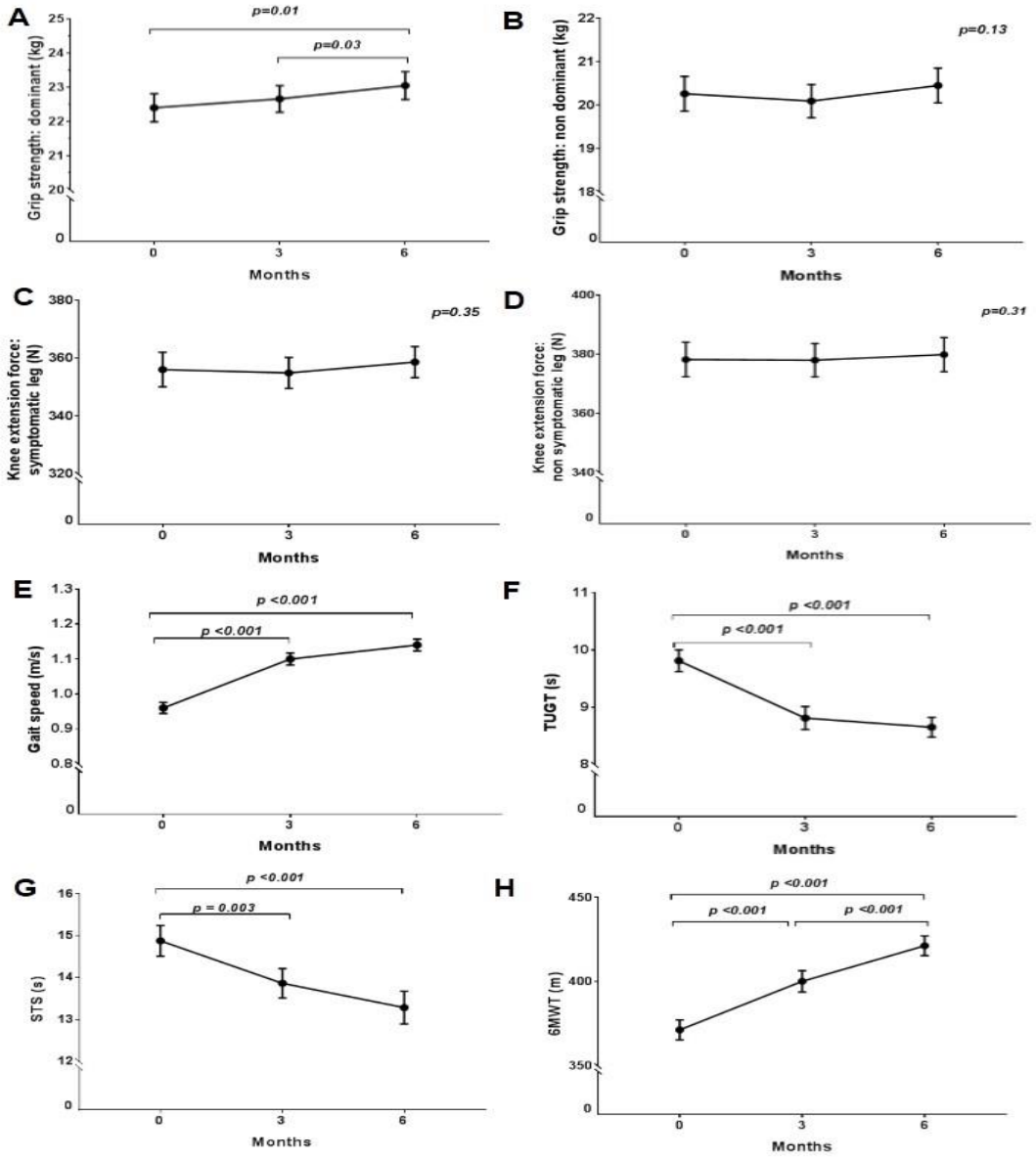


Figure 4.6 Muscle strength and physical performance at baseline, 3 and 6 months after vitamin D_2 supplementation, **A)** grip strength of dominant side **B)** grip strength of non-dominant side, **C)** knee extension force of symptomatic leg, **D)** knee extension force of non-symptomatic leg, **E)** gait speed, **F)** TUGT, **G)** STS and **H)** 6MWT.

2.3. Effects of vitamin D_2 supplementation on knee OA patients with sarcopenia after 40,000 IU vitamin D_2 supplementation

At baseline, the prevalence of sarcopenia, pre-sarcopenia, and non-sarcopenia were 8.00% (n=14), 12.60% (n=22), and 79.40% (n=139), respectively. After 40,000 IU vitamin D_2 supplementation for 6 months, the prevalence of sarcopenia decreased to 4.00% and prevalence of pre-sarcopenia increased to 16.60%. This study demonstrated that vitamin D supplementation in knee OA patients improved muscle strength and physical performance which were observed in sarcopenia patients. These patients improved to pre-sarcopenia from sarcopenia after they received vitamin D treatment They demonstrated low muscle mass but normal muscle strength and physical performance. Finally, the prevalence of non-sarcopenia was 79.40% as presented in **Figure 4.7.**

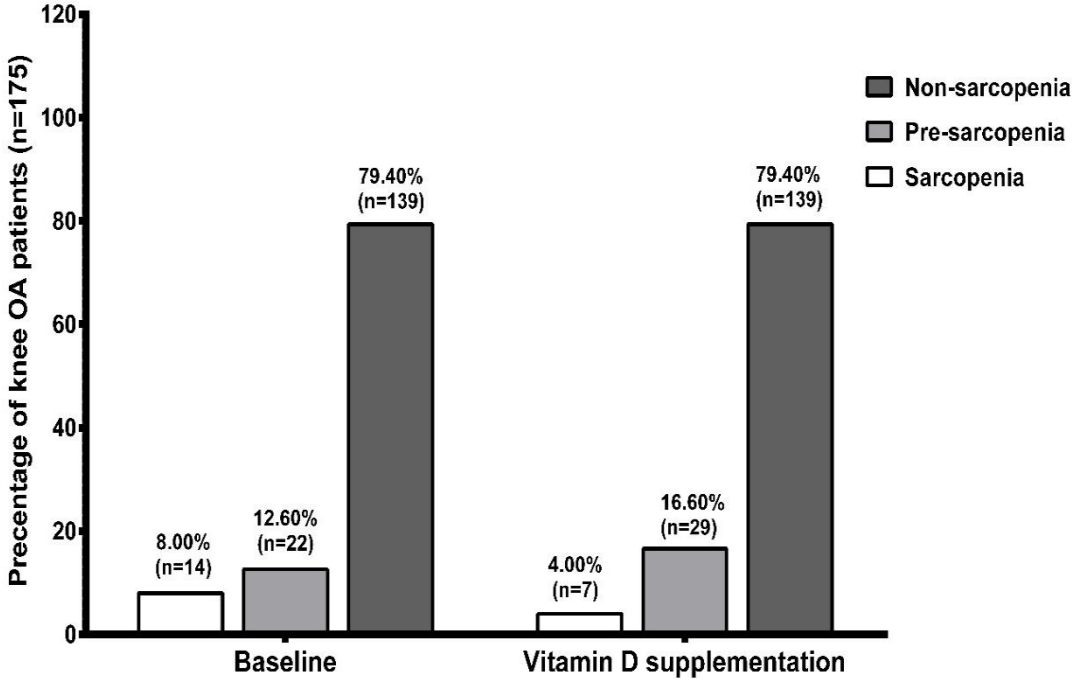


Figure 4.7 The prevalence of sarcopenia at baseline and after 40,000 IU vitamin D_2 supplementation for 6 months

2.4 Effects of vitamin D_2 supplementation on self-report pain, health-related quality of life and physical activity.

WOMAC scores and physical activity questionnaire (PAQ-EJ) did not change, whereas VAS significantly decreased (3.96±0.17 vs 3.44±0.17, p = 0.002) after treatment. Moreover, the PCS of SF-12 significantly improved (38.26±0.65 vs 40.24±0.67, p = 0.005) as shown in **Table 4.11**

Table 4.11 VAS, WOMAC and PAQ-EJ scores after vitamin D_2 supplementation

	Vitamin D_2 supplementation (n=175)			
	Mean ± SEM		Mean Difference (95% CI)	*p*
	Before	After		
VAS (0-10)	3.96±0.17	3.44±0.17	-0.51 (-0.83 to -0.19)	0.002
WOMAC				
Pain (0-10)	2.45±0.15	2.59±0.15	0.14 (-0.15 to 0.44)	0.33
Stiffness (0-10)	2.56±0.18	2.26±0.16	-0.29 (-0.62 to 0.03)	0.08
Physical disability (0-10)	2.90±0.15	2.76±0.15	-0.14 (-0.41 to 0.13)	0.31
Total score (0-10)	2.80±0.13	2.78±0.13	-0.01 (-0.08 to 0.06)	0.75
SF-12				
PCS (0-100)	38.26±0.65	40.24±0.67	1.98 (0.60 to 3.36)	0.005
MCS (0-100)	50.00±0.70	49.57±0.66	-0.42 (-1.82 to 0.97)	0.54
Physical activity				
PAQ-EJ (MET hours/week)	52.28±2.83	53.29±3.08	1.00 (-5.11 to 7.13)	0.74

VAS: Visual analogue scale, WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index, SF-12: 12-Item short form health survey, PCS: Physical health composite scores, MCS: Mental health composite scores, PAQ-EJ: physical activity questionnaire for elderly Japanese in Thai version and MET: metabolic equivalent of task.

2.5 Effects of vitamin D_2 supplementation on metabolic risk factors

The results of metabolic profile and biochemical markers were shown in **Table 4.12**. HDL cholesterol levels were elevated but there were not statistically different (55.30±1.00 vs 57.40±1.28 mg/dl, p = 0.05). In addition, LDL cholesterol levels was significantly decreased after vitamin D_2 supplementation (135.42±2.76 vs 127.64±2.78 mg/dl, p = 0.001).

Waist circumference (87.87±0.73 vs 87.82±0.71, p = 0.87), fasting blood glucose (98.06±1.30 vs 98.49±1.56, p = 0.75), insulin (5.32±0.41 vs 5.99±0.41, p = 0.13), HOMA-IR (1.34±0.11 vs 1.55±0.13, p = 0.13), total cholesterol (211.94±2.93 vs 212.90±3.14, p = 0.72), triglyceride (126.34±4.16 vs 123.70±4.63, p = 0.49), SBP (131.02±0.77 vs 131.00±0.81, p = 0.93) and DBP (78.57±0.51 vs 78.25±0.55, p = 0.19) were not different between before and after vitamin D_2 supplementation.

Table 4.12 Metabolic profile in knee OA patients after vitamin D_2 supplementation

	Vitamin D_2 supplementation (n=175)			
	Mean ± SEM		Mean Difference (95% CI)	*p*
	Before	After		
Waist circumference (cm)	87.87±0.73	87.82±0.71	-0.05 (-0.66 to 0.56)	0.87
FBG (mg/dl)	98.06±1.30	98.49±1.56	0.42 (-2.26 to 3.12)	0.75
Insulin (μU/ml)	5.32±0.41	5.99±0.41	0.66 (-0.20 to 1.53)	0.13
HOMA-IR	1.34±0.11	1.55±0.13	0.20 (-0.06 to 0.46)	0.13
Total cholesterol (mg/dl)	211.94±2.93	212.90±3.14	0.95 (-4.35 to 6.26)	0.72
HDL cholesterol (mg/dl)	55.30±1.00	57.40±1.28	2.09 (-0.03 to 4.23)	0.05
Triglyceride (mg/dl)	126.34±4.16	123.70±4.63	-2.63 (-10.32 to 5.04)	0.49
LDL cholesterol (mg/dl)	135.42±2.76	127.64±2.78	-7.77 (-12.43 to -3.12)	0.001
SBP (mmHg)	131.02±0.77	131.00±0.81	-0.77 (-0.66 to 0.60)	0.93
DBP (mmHg)	78.57±0.51	78.25±0.55	-0.81 (-0.80 to 0.16)	0.19

FBG: Fasting blood glucose, HOMA-IR: Homeostatic model assessment of insulin resistance, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, SBP: Systolic blood pressure and DBP: Diastolic blood pressure.

2.6 Effects of vitamin D_2 supplementation on vitamin D status, calcium and phosphorus homeostasis

After 40,000 IU of vitamin D_2 supplementation for 6 months, there were significantly increased in both 25(OH)D levels (20.73±0.36 vs 32.14±0.59 ng/ml, $p < 0.001$) and serum calcium levels (9.25±0.03 vs 9.34±0.04 mg/dl, $p = 0.003$). Whereas, PTH levels significantly decreased after vitamin D supplementation (53.20±1.72 vs 46.63±2.21 pg/ml, $p = 0.005$). During treatment, three OA patients (1.71%) developed mild hypercalcemia (Ca >10.5 mg/dl).

Albumin levels (4.29±0.01 vs 4.27±0.01 mg/dl, $p = 0.19$) and phosphorus (3.62±0.03 vs 3.69±0.03 mg/dl, $p = 0.10$) were not different between before and after vitamin D_2 supplementation (**Table 4.13).**

Table 4.13 Vitamin D, calcium and phosphorus homeostasis in knee OA patients after vitamin D_2 supplementation

	Vitamin D_2 supplementation (n=175)			
	Mean ± SEM		Mean Difference (95% CI)	*p*
	Before	After		
25 (OH)D (ng/ml)	20.73±0.36	32.14±0.59	11.41(10.27 to 12.54)	<0.001
PTH (pg/ml)	53.20±1.72	46.63±2.21	-6.57 (-11.08 to -2.05)	0.005
Albumin (mg/dl)	4.29±0.01	4.27±0.01	-0.01 (-0.03 to 0.007)	0.19
Calcium (mg/dl)	9.25±0.03	9.34±0.04	0.09 (0.006 to 0.18)	0.03
Phosphorus (mg/dl)	3.62±0.03	3.69±0.03	0.06 (-0.01 to 0.13)	0.10

25(OH)D: 25-hydroxyvitamin D and PTH: parathyroid hormone

2.7 Effects of vitamin D_2 supplementation on inflammation, adipokine and oxidative stress

Levels of hs-CRP (1.97±0.20 vs 2.61±0.34 mg/dl, p = 0.07), IL-6 (20.59±4.52 vs 22.37±2.32 pg/ml, p = 0.64), and leptin (25.93±1.57 vs 24.68±1.45 ng/ml, p = 0.35) were not different between before and after vitamin D2 supplementation

Levels of protein carbonyls (0.79±0.04 vs 0.70±0.03 nmol/mg, $p < 0.05$) was significantly decreased after vitamin D_2 supplementation (**Table 4.14**).

Table 4.14 Levels of inflammation, adipokine and oxidative stress in knee OA patients after vitamin D_2 supplementation

	Vitamin D_2 supplementation (n=175)			
	Mean ± SEM		Mean Difference (95% CI)	*p*
	Before	After		
hs-CRP (mg/dl)	1.97±0.20	2.61±0.34	0.64 (-0.06 to 1.35)	0.07
IL-6 (pg/ml)	20.59±4.52	22.37±2.32	1.78 (-5.75 to 9.31)	0.64
Leptin (ng/ml)	25.93±1.57	24.68±1.45	-1.24 (-3.89 to 1.39)	0.35
Protein carbonyls (nmol/mg)	0.79±0.04	0.70±0.03	-0.08 (- 0.16 to -0.003)	0.04

hs-CRP: high-sensitivity C-reactive protein, IL-6: interleukin-6

Part III Association of vitamin D and serum leptin vs all parameters in knee OA patients.

3.1 Serum vitamin D

3.1.1 Association of 25(OH)D and body composition

Association between 25(OH)D and body composition was performed by Spearman's correlation analysis. BMI and fat mass were not associated with 25(OH)D levels at baseline (r = 0.02, *p* = 0.78).

After vitamin D_2 supplementation, levels of 25(OH)D were negatively correlated with BMI (r = - 0.24, *p* = 0.002) and fat mass (r = - 0.20, *p* = 0.008) as show in **Figure 4.8**

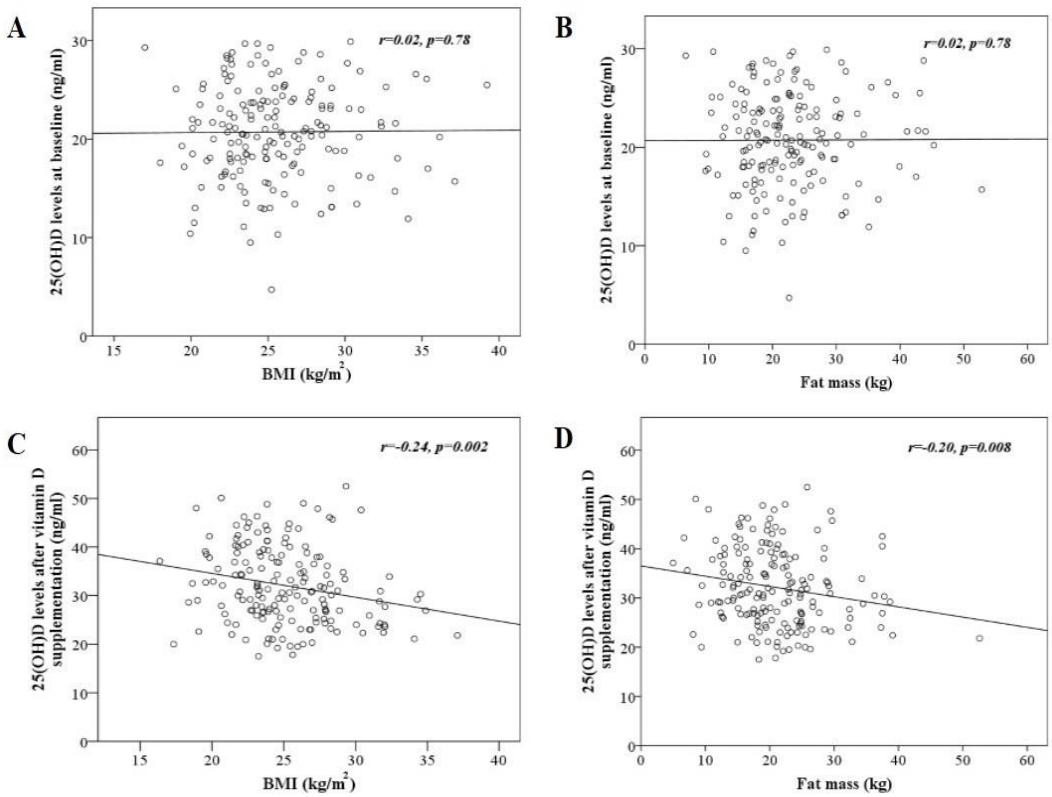


Figure 4.8 Association of vitamin D levels and body composition before and after vitamin D supplementation **A)** and **B)** BMI and fat mass at baseline **C)** and **D)** BMI and fat were negatively associated with 25(OH)D levels in knee OA patients with vitamin D_2 supplementation.

3.1.2 Association of 25(OH)D levels, muscle strength and physical performance in knee OA patients

Grip strength was not correlated with 25(OH)D levels (r =0.03, p = 0.56) but knee extension force both in symptomatic leg (r = 0.25, p < 0.001) and non-symptomatic leg (r = 0.31, p < 0.001) were positively associated with levels of 25(OH)D as shown in **Figure 4.9**.

No correlation between 25(OH)D levels and gait speed (r = 0.06, p = 0.33), TUTG (r = -0.05, p = 0.39) and STS (r = -0.02, p = 0.69) was observed. Whereas, there was a weak relationship between 25(OH)D levels and 6MWT (r = 0.14, p = 0.03)

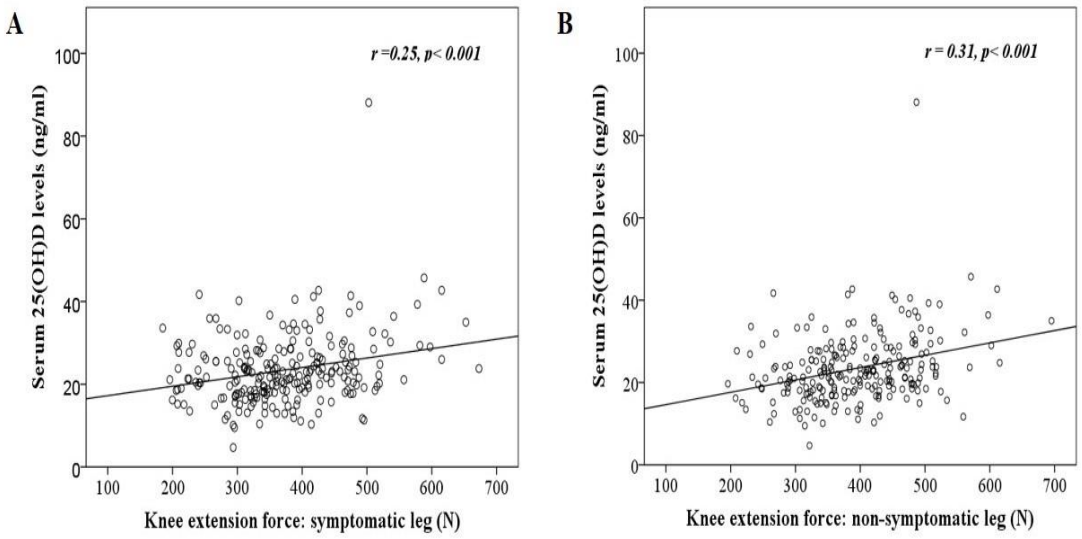


Figure 4.9 Association of vitamin D levels and knee extension force **A)** symptomatic leg and **B)** non-symptomatic leg

3.1.3 Association of vitamin D levels and biochemical markers in knee OA patients

Association between serum 25(OH)D and biochemical markers such as leptin, IL-6, protein carbonyl, and PTH levels are presented in **Figure 4.10.** Leptin (r = -0.26, $p < 0.001$), Il-6 (r = -0.32, $p < 0.001$), protein carbonyls (r = -0.28, $p < 0.001$) and PTH (r = - 0.31, $p < 0.001$) were negatively correlation with serum 25(OH)D concentrations in OA patients.

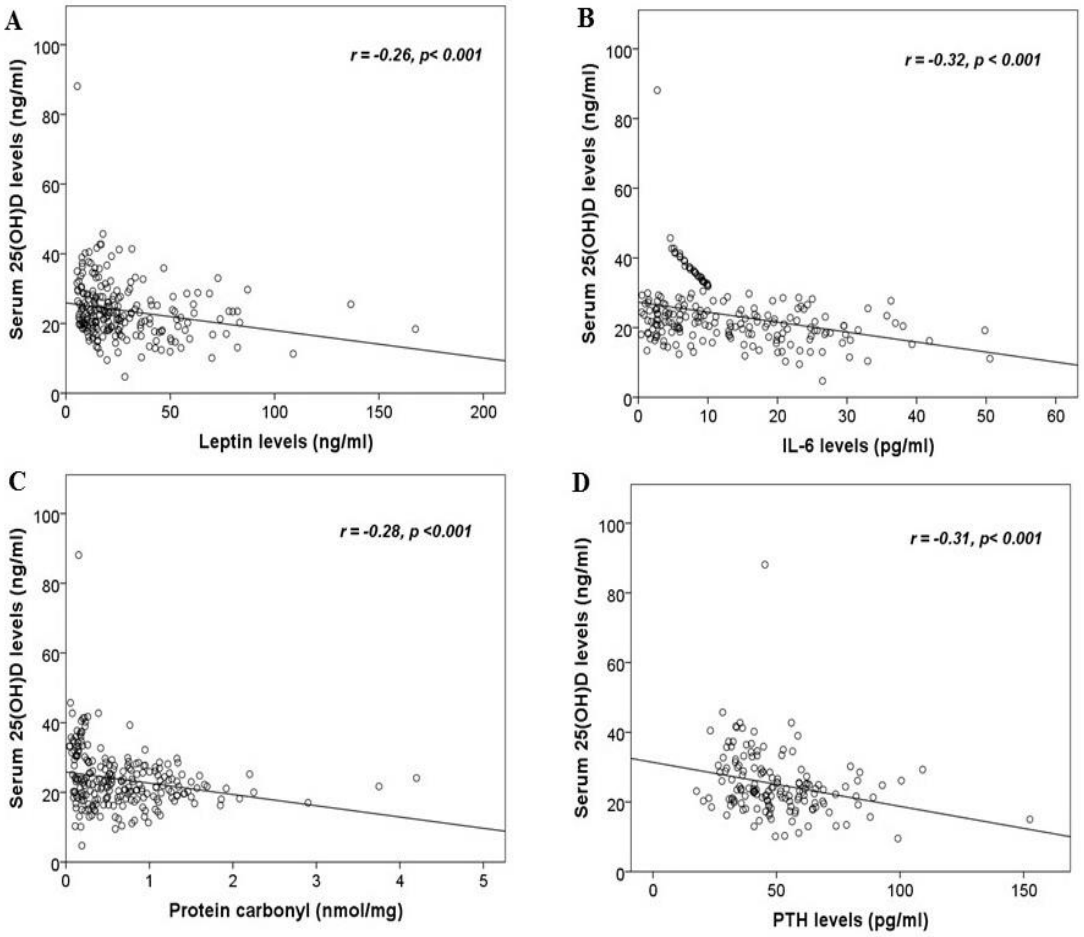


Figure 4.10 Association between serum 25(OH)D levels and biochemical markers **A)** leptin, **B)** IL-6, **C)** protein carbonyl, and **D)** PTH.

3.1.4 Multiple linear regression models of serum 25(OH)D levels

In the multivariable models of 25(OH)D levels, after adjustment for age, sex, BMI and fat mass, serum 25(OH)D levels were positively associated with knee extension force (ß =0.26, $p < 0.001$) and 6MWT (ß =0.15, $p = 0.02$). There were negatively association with leptin (ß = -0.16, $p = 0.03$), PTH (ß = -0.24, $p = 0.003$), IL-6 (ß = - 0.36, $p < 0.001$), and protein carbonyl (ß = -0.15, $p = 0.01$) as shown in **Table 4.15.**

Table 4.15 Multiple linear regression models of serum 25(OH)D levels

	Multivariable*		
	ß	**Standard Error**	***p***
Biomarker			
Insulin (µU/ml)	0.05	0.19	0.50
HOMA-IR	0.05	0.65	0.56
Leptin (ng/ml)	-0.16	0.03	0.03
PTH (pg/ml)	-0.24	0.04	0.003
hs-CRP (mg/dl)	0.01	0.23	0.85
IL-6 (pg/ml)	-0.36	0.05	<0.001
Protein carbonyl (nmol/mg)	-0.15	0.93	0.01
Body composition			
SMI (%)	0.10	0.27	0.41
Visceral fat rating	0.35	0.48	0.11
Muscle strength			
Grip strength (kg)	0.02	0.13	0.75
Knee extension force (N)	0.26	0.006	<0.001
Physical performance			
Gait speed (m/s)	0.06	2.71	0.34
TUGT (s)	-0.05	0.22	0.42
STS (s)	-0.01	0.12	0.84
6MWT (m)	0.15	0.007	0.02

25(OH)D: 25-hydroxyvitamin D, hs-CRP: high-sensitivity C-reactive protein, TUGT: Timed up and go test, STS: sit to stand, and 6MWT: Six-Minute Walk test.

* Adjusted for age, sex, BMI and fat mass

3.2 Serum leptin

3.2.1 Association of serum leptin and body composition in knee OA patients

Association between serum leptin levels, BMI, WC, fat mass, visceral fat rating, SMI and ASMI were tested by Spearman's correlation analysis as presented in **Figure 4.11.**

BMI ($r = 0.62$, $p < 0.001$), WC ($r = 0.52$, $p < 0.001$), fat mass ($r = 0.71$, $p < 0.001$) and visceral fat rating ($r = 0.49$, $p < 0.001$) showed positive correlation with serum leptin concentrations in OA patients.

In muscle mass index, SMI presented a negative correlation ($r = -0.64$, $p < 0.001$), while ASMI was not associated with leptin levels ($r = 0.14$, $p = 0.10$), suggesting that muscle mass per body weight was correlated with leptin levels.

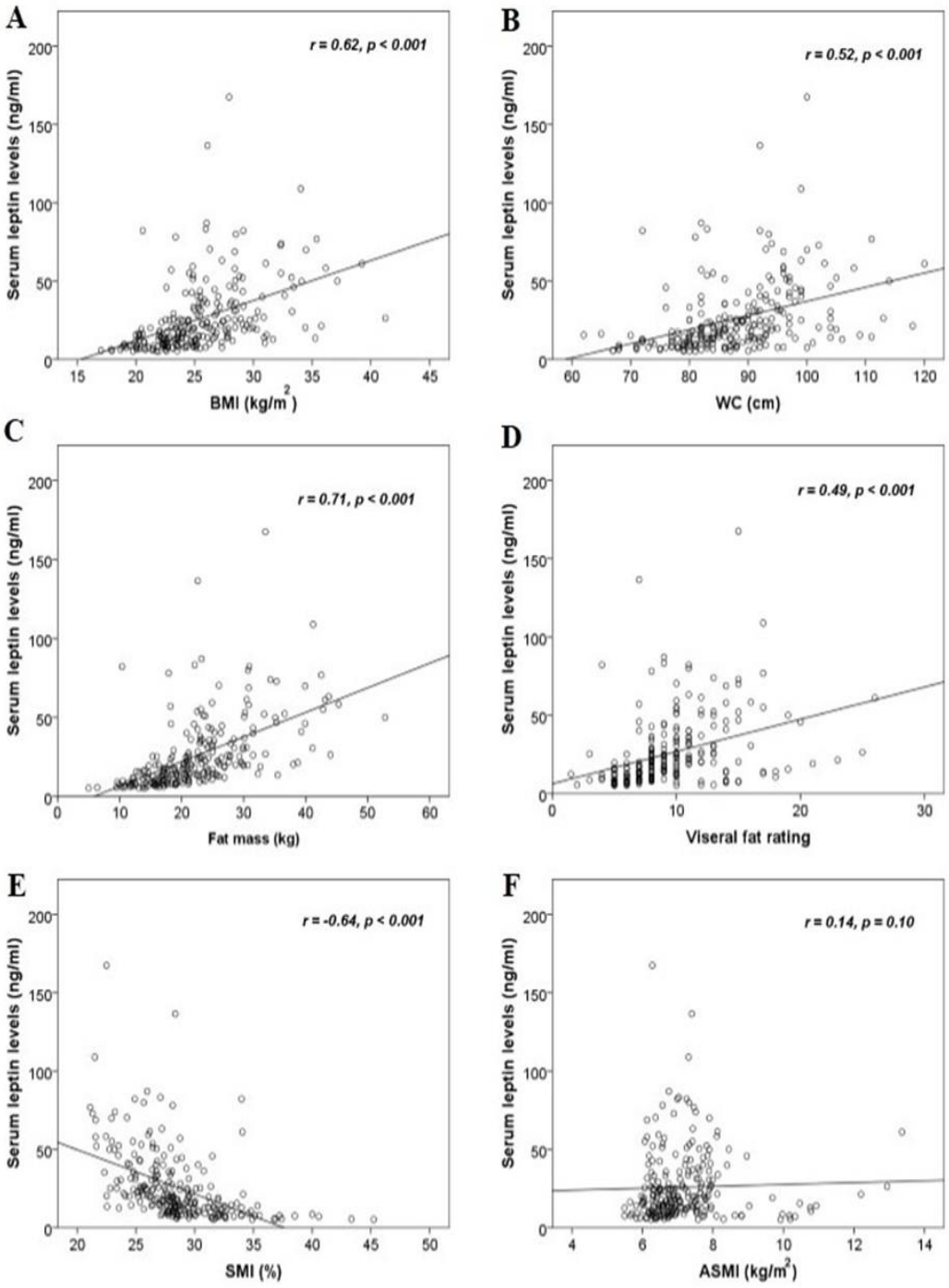


Figure 4.11 Scatter diagram and correlation analysis of serum leptin levels with **A)** BMI, **B)** WC, **C)** Fat mass, **D)** Visceral fat rating, **E)** SMI, and **F)** ASMI in knee OA patients.

3.2.2 Association of serum leptin levels, muscle strength and physical performance in knee OA patients

No correlation between leptin levels and muscle strength was observed ($p > 0.05$). However, there was a weak relationship between serum leptin and physical performance. Serum leptin was also positively associated with TUGT ($r = 0.27$, $p < 0.001$) and STS ($r = 0.27$, $p < 0.001$). Additionally, leptin levels were negatively correlated with gait speed ($r = -0.25$, $p < 0.001$) and 6MWT ($r = -0.24$, $p < 0.001$) as shown in **Figure 4.12.**

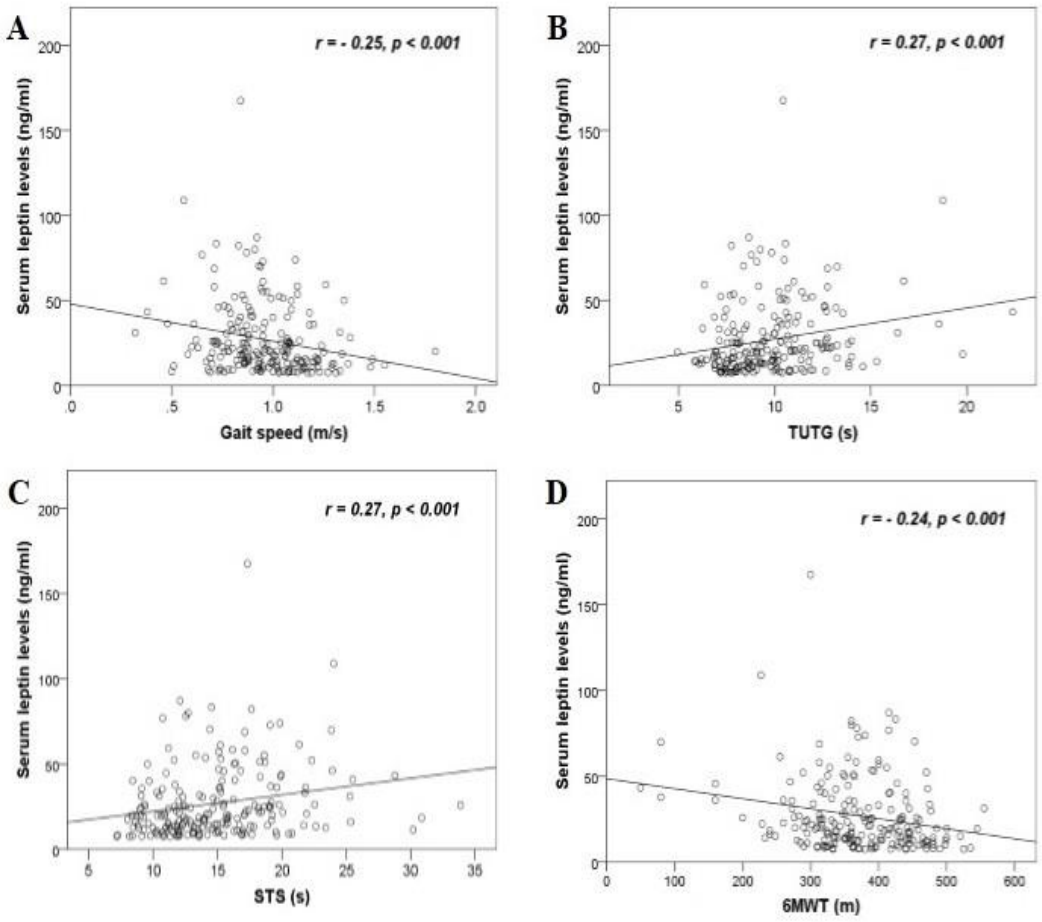


Figure 4.12 Association between serum leptin levels and physical performance tests **A, D)** Gait speed and 6MWT were negatively associated with serum leptin levels. **B, C)** TUGT and STS were positively correlated with serum leptin levels in knee OA patients.

3.2.3 Association of serum leptin levels and biomarkers in knee OA patients

There was a positive association between leptin levels and metabolic risk factors. Serum leptin was associated with insulin (r = 0.34, p < 0.001), HOMA-IR (r = 0.34, p < 0.001) and a weak relationship with triglyceride (r = 0.23, p = 0.001). Moreover, leptin levels were positively associated with hs-CRP (r= 0.35, p < 0.001) as presented in **Figure 4.13**.

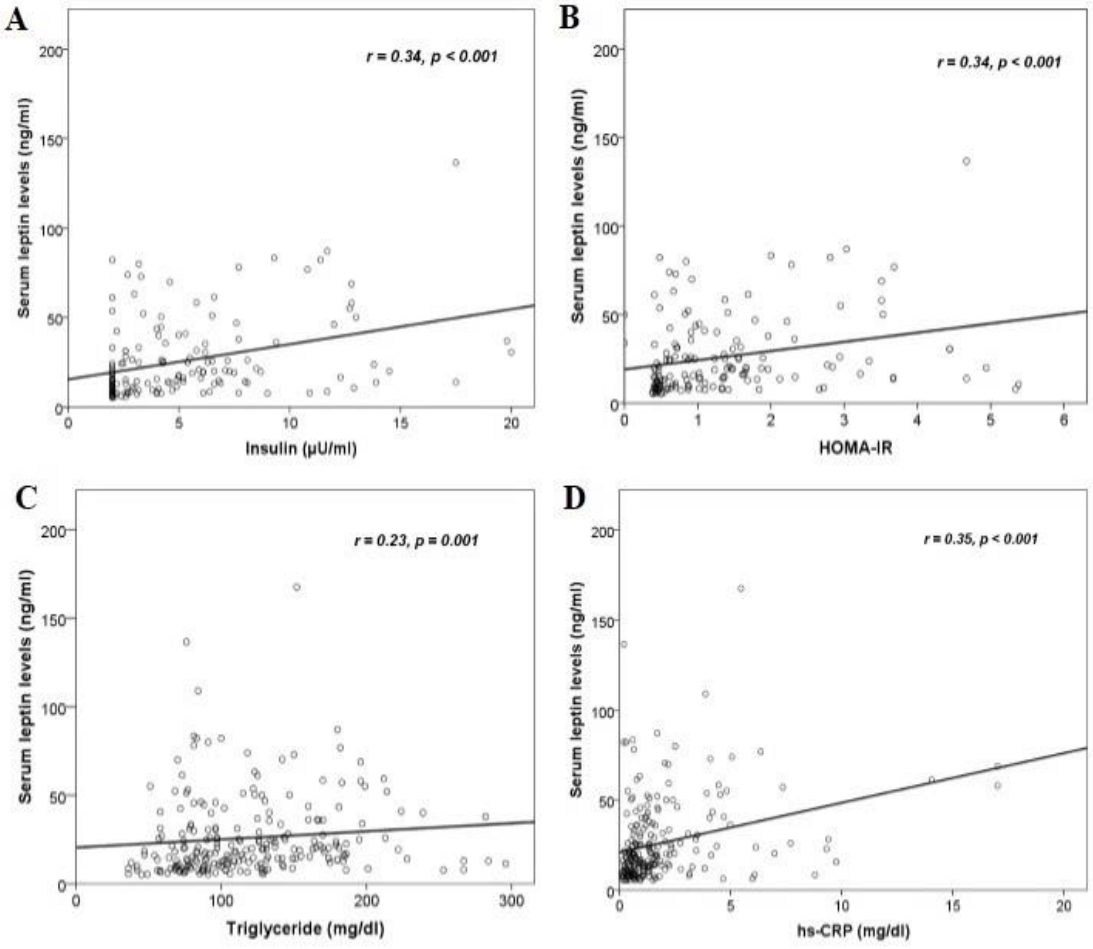


Figure 4.13 A positive association between serum leptin levels, metabolic risk factors and inflammation **A)** insulin, **B)** HOMA-IR, **C)** triglyceride and **D)** hs-CRP

3.2.4 Multiple linear regression models of serum leptin levels

In the multivariable models of leptin levels, after adjustment for age, sex, BMI and fat mass, serum leptin levels were positively associated with insulin (ß =0.17, $p < 0.05$), hs-CRP (ß =0.14,$p < 0.05$), and visceral fat rating (ß =0.62, $p < 0.01$). There were negatively association with 25(OH)D (ß = -0.16, $p < 0.01$), SMI (ß = -0.26, $p < 0.05$), Grip strength (ß = - 0.17, $p < 0.05$), knee extension force (ß = - 0.12, $p < 0.05$) and 6MWT (ß = -0.14, $p < 0.05$) as shown in **Table 4.16.**

Table 4.16 Multiple linear regression models of serum leptin levels

	Multivariable*		
	ß	**Standard Error**	***p***
Biomarker			
Insulin (μU/ml)	0.17	0.36	0.01
HOMA-IR	0.12	1.18	0.08
25(OH)D (ng/ml)	- 0.12	0.15	0.03
PTH (pg/ml)	-0.02	0.08	0.74
hs-CRP (mg/dl)	0.14	0.46	0.02
IL-6 (pg/ml)	0.005	0.02	0.93
Protein carbonyl (nmol/mg)	- 0.43	2.17	0.44
Body composition			
SMI (%)	- 0.26	0.70	0.02
Visceral fat rating	0.62	1.28	0.009
Muscle strength			
Grip strength (kg)	- 0.17	0.29	0.03
Knee extension force (N)	- 0.18	0.01	0.04
Physical performance			
Gait speed (m/s)	- 0.10	6.18	0.11
TUGT (s)	0.07	0.51	0.23
STS (s)	0.09	0.27	0.09
6MWT (m)	- 0.14	0.017	0.02

TUGT: Timed up and go test, STS: sit to stand, and 6MWT: Six-Minute Walk test.

* Adjusted for age, sex, BMI and fat mass

CHAPTER V

Discussion

Osteoarthritis is considered as a degenerative joint disorder that leads to musculoskeletal disability and pain. OA co-exists frequently with vitamin D deficiency. The current study aimed to investigate prevalence rate of vitamin D deficiency in knee OA and to analyze the relationship between various biomarkers, muscle strength and physical performance in knee OA patients. Subsequently, we further determined the efficacy of ergocalciferol (vitamin D_2) supplementation on muscle mass, muscle strength, and physical performance in knee OA patients with low vitamin D status.

The results showed that knee OA patients had highly prevalent vitamin D insufficiency (49.60%), vitamin D deficiency (33.60%) and vitamin D sufficiency (16.80%). In addition, knee OA patients with vitamin D deficiency were younger than vitamin D sufficiency group. The results of this study corroborated with previous studies which reported that 81.70% of OA patients were vitamin D deficiency (98) and 63.00% of primary knee OA had low vitamin D status (13). Heidari et al. found the association between vitamin D deficiency and knee OA of aged less than 60 years (14). Knee OA patients need to have vitamin D treatment to improving vitamin D levels. due to their low vitamin D status;

In this regard, we observed that knee OA patients with vitamin D deficiency had lower knee extension force than vitamin D sufficiency group but grip strength and physical performance were not different. These results were in agreement with Barker's study showing that knee OA with vitamin D deficiency had quadriceps strength impairment (16). Furthermore, our study reported that the prevalence of sarcopenia in vitamin D sufficiency group was 12.50% (n=5), sarcopenia in vitamin D insufficiency and deficiency group were 11.10% (n=13) and 7.40% (n=6), respectively. As a result, the prevalence of sarcopenia among vitamin D status groups were not different because of small numbers of subgroup in this study. There was no finding of the association between vitamin D deficiency and sarcopenia.

Self-report pain, health-related quality of life, physical activity and frequency of falls in knee OA patients were not different between vitamin D deficiency and

sufficiency groups. These results were in line with previous studies, Lee et al. reported that 25(OH)D levels was not significantly associated with severity of knee pain in elderly (101). In contrast, Laslett et al. found that moderate vitamin D deficiency could predict changes in knee pain over 5 years by WOMAC (99).

Metabolic risk factors and MetS in this study demonstrated that knee OA patients with vitamin D deficiency had significantly higher levels of LDL cholesterol and triglyceride than those with vitamin D insufficiency and sufficiency group. Data from the studies of Wang et al. (129) and Park and Lee (130) suggested that serum 25(OH)D levels were inversely associated with LDL cholesterol and triglyceride., Zhang et al found that Chinese adults with low levels of 25(OH)D had high levels of LDL cholesterol (129). However, vitamin D influences lipid profile is unclear. Previous study has been suggested that the optimal vitamin D levels increased level of intestinal calcium absorption that it could decrease intestinal absorption of fatty acid because of the formation of insoluble calcium-fatty complexes. Finally, LDL cholesterol and triglyceride absorption could be decreased in intestinal (131). Therefore, these results showed that vitamin D deficiency may be associated with the increased risk of dyslipidemias.

Results of vitamin D and calcium homeostasis demonstrated that PTH levels in vitamin D deficiency group were significantly higher than vitamin D sufficiency group. The previous study reported that low vitamin D status was associated with the elevated bone turnover by increasing PTH levels (132). Moreover, high levels of PTH are related with the risk of fall, fracture, poorer outcomes in terms of frailty (11) because PTH action stimulates the transformation of proosteoclasts into mature osteoclasts that lead to increasing bone turnover (86). Vitamin D deficiency stimulates PTH production that leads to secondary hyperparathyroidism. In clinical, higher levels of PTH can cause the symptoms of muscle weakness and fatigue (133). *In vivo*, PTH has been shown to induce protein catabolism by the release of both alanine and glutamine from rat skeletal muscle (134), reduced both mitochondrial oxygen consumption and the oxidation of long-chain fatty acids which interfered the usage of an important source of energy for skeletal muscle (135). Consequently, optimal

vitamin D levels may help improve skeletal muscle function, reduce the risk of fall, fracture, and osteoporosis.

Levels of Inflammation cytokine (IL-6), adipokine (leptin) and oxidative stress biomarker (protein carbonyls) in knee OA patients with vitamin D deficiency were higher than those observed in vitamin D sufficiency group. Vitamin D deficiency also affects on several organ and metabolic process. Several studies reported that low vitamin D status was associated with high level of fasting blood glucose, decreased insulin sensitivity, increased risk of diabetes mellitus and cardiovascular (136). Low levels of 25(OH)D related with obesity due to the increased fat accumulation in the body and retention of vitamin D in those fat stores. Accordingly, 25(OH)D deficiency in the circulation results in stimulation of PTH and synthesis of 1α hydroxylase to maintenance of 1,25$(OH)_2$D. It was well known that high body fat mass is related to an increasing levels of leptin in the circulation (137). Similar to our results, patients with vitamin D deficiency had higher leptin concentrations compared with individuals with normal vitamin D status (138). Furthermore, the relationship between the inflammatory cytokine IL-6 and vitamin D has been previously demonstrated. Vitamin D deficiency could result in immune dysfunction such as impaired phagocytosis, chemotaxis, and elevated production of proinflammatory cytokines, particularly, IL-6 and TNF-α (136). Oxidative stress is one of factors leading to ageing and degenerative diseases which oxidative metabolism generates reactive oxygen species (ROS). There is altering and damaging cell components. Previous studies have shown that increasing protein oxidation might contribute to loss of muscle strength and physical function in older adults (84, 85). Moreover, protein carbonyl has been implicated in the pathogenesis of sarcopenia or low muscle mass (139). As a result, protein carbonyl reactions were suspected of involvement in the systemic effects of skeletal muscle dysfunction by reducing ATP production, decreasing muscle type II fibers, muscle contraction and endurance (140, 141).

Our findings support vitamin D deficiency lead to poor lower limb muscle strength, increase risk of metabolic risk factors and bone turnover, and enhance production of IL-6, leptin and protein carbonyls. Therefore, vitamin D plays an important role of systemic metabolic effects in knee OA patients.

Accordingly, we further determine the efficacy of vitamin D_2 supplementation on muscle mass, muscle strength, and physical performance in knee OA patients with low vitamin D status. After six months supplementation of 40,000 IU of vitamin D_2 per week, 57% (n=100) participants achieved vitamin D sufficiency whereas 40% and 3% participants had vitamin D insufficiency and deficiency, respectively. These data demonstrated the effect of 40,000 IU/wk vitamin D_2 for succeed optimal vitamin D levels. They have possibility to achieving vitamin D sufficiency from 38.90% (n=28) of vitamin D deficiency group and 69.90% (n=72) of vitamin D insufficiency group after vitamin D supplementation. There was only 6.90% (n=5) of vitamin D deficiency group and 30.10% (n=31) of vitamin D insufficiency group remained at the same vitamin D levels. This is similar to that reported previously in patients with MetS in Thailand who were treated with ergocalciferol 40,000 IU/wk for 8 weeks (n=30). After vitamin D_2 supplementation, 60% of participants had normal vitamin D levels, 27% had vitamin D insufficiency and 13% of these had vitamin D deficiency (114).

The body composition such as, weight, percentage of fat, fat mass and visceral fat rating after vitamin D_2 supplementation decreased significantly than their baseline values but skeletal muscle mass did not change. Our results showed that the participants had weight loss which might due to change of their lifestyles, and improved significantly physical function due to increasing physical health composite scores (PCS) of SF-12 while physical activity assessments from PAQ-EJ did not differ.

Six months of 40,000 IU/wk vitamin D_2 supplementation significantly improved grip strength and physical performance but did not improve knee extension force. Moreover, knee OA with sarcopenia who received vitamin D_2 treatment increased grip strength and physical performance. They turned into pre-sarcopenia i.e., low muscle mass but normal muscle strength and physical performance. Finally, vitamin D supplementation might reduce the prevalence of sarcopenia in knee OA patients. In this respect, our results are consistent with the findings of Zhu et al. who reported that hip muscle strength and TUGT improved significantly after 1,000 IU/d vitamin D_2 supplementation for 1 year in older women with vitamin D insufficiency (142). Lagari et al. presented that vitamin D_3 supplementation might be most beneficial in older populations with poor physical function (118). Therefore, vitamin D supplementation

was found to increase muscle function in those who were weak and slow at baseline. In addition, previous studies showed that vitamin D supplementation significantly improved lower limb muscle strength, grip strength, and timed up & go test and reduced fall rates (17, 19). Regarding the effect of vitamin D on skeletal muscle, Sato et al. found that the mean of type II muscle fiber diameter and percentage of type II fibers increased significantly after 1,000 IU/day vitamin D_2 treatment over 2 years in post-stroke hemiplegia in the elderly (20). Ceglia, et al. suggested that intramyonuclear vitamin D receptor (VDR) concentration increased 30% and total (type I and II) muscle fiber size increased 10% after vitamin D_3 supplementation in mobility-limited elderly women (21).

On the contrary, several studies have reported that vitamin D supplementation did not improve muscle strength or physical function. Kenny et al. have shown that vitamin D supplementation did not improve muscle strength or physical function in a group of healthy community-dwelling older men (22). Oosterwerff et al. revealed that moderate-dose vitamin D_3 supplementation (1,200 IU/day) did not significantly improve six-minute walk test results or physical performance in overweight and non-Western immigrants (23). In OA patients, vitamin D supplementation did not improve physical function (24). In fact, the action of vitamin D on skeletal muscle can also occur via the $1,25(OH)_2D$ receptor or the vitamin D receptor (VDR). Increased VDR content in muscle cells after vitamin D treatment (21) would positively affect muscle contraction by modulating muscle cell calcium influx by altering the activity of calcium pumps via a calcium binding protein and calbindin D9k, which are located in the sarcoplasmic reticulum and sarcolemma (5). Furthermore, vitamin D supplementation increased the proportion of type II fibers (20, 21). However, it remains uncertain if the increase in the proportion of type II fibers are resulted from the formation of new type II fibers or the transformation of previously existing fibers from type I to type II (143). *In vitro* experiments, $1,25(OH)_2D$ motivated muscle cell differentiation through the regulation of several growth factors and inhibitors. The administration of $1,25\ (OH)_2D$ to C2C12 myoblasts increased myogenic differentiation by up-regulated expression of insulin-like growth factor II (IGF-II) and follistatin and up-regulated expression of myostatin (93). Moreover, the treatment of C2C12 muscle cells with $1,25\ (OH)_2D$ enhanced vascular

endothelial growth factor alpha (VEGFa) and fibroblast growth factor-1 (FGF-1) which both factors involve angiogenesis, tissue healing and muscle myogenesis (94). Furthermore, this study suggest that vitamin D_2 supplementation significantly improve grip strength and physical performance in knee OA with low vitamin D status.

Self-report pain and health-related quality of life showed improvement after vitamin D supplementation according to results obtained from visual analog score (VAS) and PCS of SF-12 questionnairs, but did not improve WOMAC score. Sanghi et al. showed that WOMAC and VAS decreased significantly after treatment (13). On the other hand, previous studies reported that vitamin D supplementation did not reduce knee pain and cartilage volume loss. Moreover, it did not improve physical function (24, 120). In this study, patients with knee OA had mild to moderate symptoms; consequently, the effect of vitamin D supplement did not affect WOMAC score. Moreover, some patients used nonsteroidal anti-inflammatory drugs (NSAIDs) for pain relief which significantly reduced VAS score.

The current study demonstrated that vitamin D treatment leaded to a significant reduction in LDL-cholesterol. The participants had weight loss, decreased the percentage of fat, fat mass and visceral fat rating that resulted in reduce LDL-cholesterol levels in this study. Moreover, some patients with hyperlipidemia use hypolipidemic drugs leading to reduce significantly LDL levels. The previous studies have shown a significant reduction in LDL- cholesterol levels after vitamin D supplementation (144, 145). Schnatz et al. reported that there was a significantly improved 25(OH)D and decreased LDL-Cholesterol levels after 1,000 mg of calcium combined with 400 IU/d vitamin D_3 over 2 years in postmenopausal women. They observed a positive correlation between 25(OH)D and HDL- Cholesterol levels and a negative correlation between 25(OH)D, LDL-Cholesterol and triglyceride levels (146). Mohamad et al. demonstrated that patients with type 2 diabetes mellitus with high concentrations of 25(OH)D >61 ng/ml after 4500 IU/d of vitamin D_3 for 2 months had significantly lower levels of total cholesterol and LDL-cholesterol than those of the low or middle percentiles of 25(OH) D levels (144). However, vitamin D supplementation did not improve lipid profiles in obese individuals (147, 148). The effects of vitamin D increase level of intestinal calcium intake which calcium may

reduce fatty acid absorption due to the formation of insoluble calcium–fatty complexes in the gut. Therefore, serum levels of LDL-cholesterol would be decreased by the reduced absorption of saturated fatty acids (131). Moreover, calcium increase the conversion of cholesterol into bile acids as a result of its potentiality to bind with bile acids that elevate cholesterol excretion (149).

Vitamin D_2 supplementation also effected on vitamin D status, calcium and phosphorus homeostasis. We found that 25(OH)D and Ca increased as well as PTH levels decreased significantly after supplementation.. Only .71% (n=3) of knee OA patents with mild hypercalcemia after vitamin D treatment was observed in this study. Pietras et al reported that no incidents of vitamin D toxicity and normal levels of serum calcium in patients who were treated with 50,000 IU of vitamin D_2 every other week for up to 6 years (150). Feldman et al. found that 14% of elderly adults in residential care who received ergocalciferol 20,000 IU/wk for 1 year had hypercalcemia, in addition, hypercalcemia was not related with higher vitamin D levels (151). Del Valle et al. studied a high-dose ergocalciferol 72,000 IU/wk for 12 weeks and maintenance therapy 24,000 IU/week during 36 weeks in hemodialysis patients. They found that only 1.8% had hypercalcemia. Therefore, our study demonstrated that 40,000 IU of vitamin D_2 supplementation was able to enhance 25(OH)D levels and safety profile with a low incidence of hypercalcemia. Moreover, this study showed a decrease of PTH concentrations of 12.34% in knee OA patients after 6 months supplement. In addition, several studies have reported vitamin D supplementation significantly reduced PTH concentrations of (152-154). Similar to our results, Zisman et al. described a decrease in PTH of 13.1% in chronic kidney disease stage 3 after ergocalciferol treatment duration of 7.4 months (154). Therefore, ergocalciferol supplementation may reduce PTH function by depressing bone turnover

No effect was also observed on hs-CRP, IL-6, and leptin. However, vitamin D treatment leaded to a significant reduction of oxidative protein damage by decreasing levels of protein carbonyl. Previous studies have shown that protein carbonyl associated with low muscle strength and poor physical performance in older adults (84, 85). These findings suggest that high levels of vitamin D after supplementation may reduce reactive oxygen species from damaging proteins.

Association of vitamin D and body composition in knee OA patients was also observed. We found a weak negative association between both 25(OHD and BMI and 25(OH)D and fat mass after vitamin D_2 supplementation. In agreement with our results, Lagari, et al. revealed that higher fat mass was related to lower vitamin D status (118). Actually, obesity is limited mobility and enhanced storage of vitamin D in adipose tissue. Wortsman et al have shown that vitamin D precursor, 7-dehydrocholesterol in the skin of obese individuals were not impaired but they produced half the amount of vitamin D produced by non-obese individuals after ultraviolet B (UVB) irradiation. On the other hand, 25(OH)D levels were not different between the obese and non-obese individuals after 50,000 IU vitamin D_2 supplementation. These results suggested that obesity might decrease the bioavailability of vitamin D due to reduction of the release of vitamin D_3 from the skin into the circulation (155). Accordingly, patients with a high BMI or obesity may also experience slower rises in serum vitamin D level than people with normal or thin body composition. Our results suggest that knee OA patients with obesity or higher BMI may need longer treatment times and higher doses of vitamin D supplementation to achieve optimal serum 25(OH)D levels.

We further observed the relationship between leptin levels, body composition, muscle strength, physical performance and biomarkers. This study showed that leptin levels were positively correlated with BMI, WC, fat mass, and visceral fat rating. Further analysis revealed that leptin levels were negatively associated with SMI index (muscle mass per body weight), while there was no correlation with ASMI (muscle mass per height). Interestingly, definitions of low muscle mass are representative the methods by using muscle mass per height squared (ASMI) or per body weight (SMI). These results corroborated with previous studies reporting that the elderly with lower skeletal muscle mass had significantly higher leptin levels than those with normal muscle mass group (156) Leptin concentrations were positively related with visceral obesity and negatively related with thigh muscle cross-sectional area in middle-aged to elderly (75). In addition, muscle mass per body weight might be better predictor for higher Framingham 10-year scores and greater risk of cardiovascular diseases than adjusted per height squared (157, 158). This finding suggests that low muscle mass defined by the SMI index may be an appropriate definition of metabolic risk factors.

The results of relationship between leptin levels, muscle strength and physical performance, showed a weak significant association between leptin levels and physical performance. After adjustment with age, sex, BMI and fat mass in a multivariate regression analysis, leptin levels were independently associated with poor muscle strength and a short distance of 6MWT. Our results seemed to corroborate with previous finding that leptin levels were negatively correlated with the modified physical performance testing, muscle strength and peak oxygen consumption (159). On the other hand, leptin levels were also correlated with poor physical functioning but were not associated with quadriceps or grip strength in midlife females (76)

Leptin concentrations were positively associated with insulin, insulin resistance (HOMA-IR) and hs-CRP but were negatively related with 25(OH) levels. Leptin promoted not only energy expenditure and prevents fatty acid accumulation in muscle but also an essential role in inflammation, angiogenesis and sarcopenia (72, 160). Previous studies reported that high levels of leptin associated with an increasing HOMA-IR index leading to insulin resistance (161) and high levels of CRP (160). Moreover, our study presented that low vitamin D status associated with an increasing leptin levels. Consequently, leptin resistance and low vitamin D status may effect on insulin sensitivity, inflammation, promote atherosclerosis and sarcopenia (161, 162).

This study had several limitations. First, the before-after design of this study did not include a control group. The lack of randomization and drug intervention of subjects potentially weaken our findings relative to the therapeutic effect of vitamin D supplementation. Second, the sample size was small and the proportion of men was low. Both of which prevented us from establishing the clinical relevance particularly the change of muscle strength. Third, we examined a marker of oxidative damage using plasma protein carbonyls which were not directly measured in skeletal muscle. Although, muscle biopsy directly measured oxidative protein damage in tissues but it was difficult to conduct in a large population study. Finally, 8.37% of patients lost the follow-up. While this rate was higher and can be considered ideal, the loss to follow-up rate in the present study was lower than loss rates reported from others.

In conclusion, our results suggested that 40,000 IU of vitamin D_2 supplementation reduced oxidative protein damage, decreased pain, improved quality

of life, and increased grip strength and physical performance. Therefore, vitamin D supplementation is a safe and inexpensive way to improve physical function in this population. Based on these findings, we suggested vitamin D supplementation at the dosage of 40,000 IU/wk in knee OA patients with poor physical function.

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APPENDIX

APPENDIX A

Sarcopenia in knee OA patients

1. The prevalence of sarcopenia in knee OA patients

In this study, 238 knee OA patients from orthopaedics outpatient clinic were enrolled. The prevalence of sarcopenia using the Asian Working Group for Sarcopenia (AWGS) suggested algorithm for sarcopenia as shown in **Figure 1.** Sarcopenia is characterized by the presence of both low muscle mass with poor physical performance or muscle strength whereas pre-sarcopenia is defined as only low muscle mass. The prevalence of sarcopenia, pre-sarcopenia, and non-sarcopenia was 10.10%, 12.60%, and 77.40%, respectively.

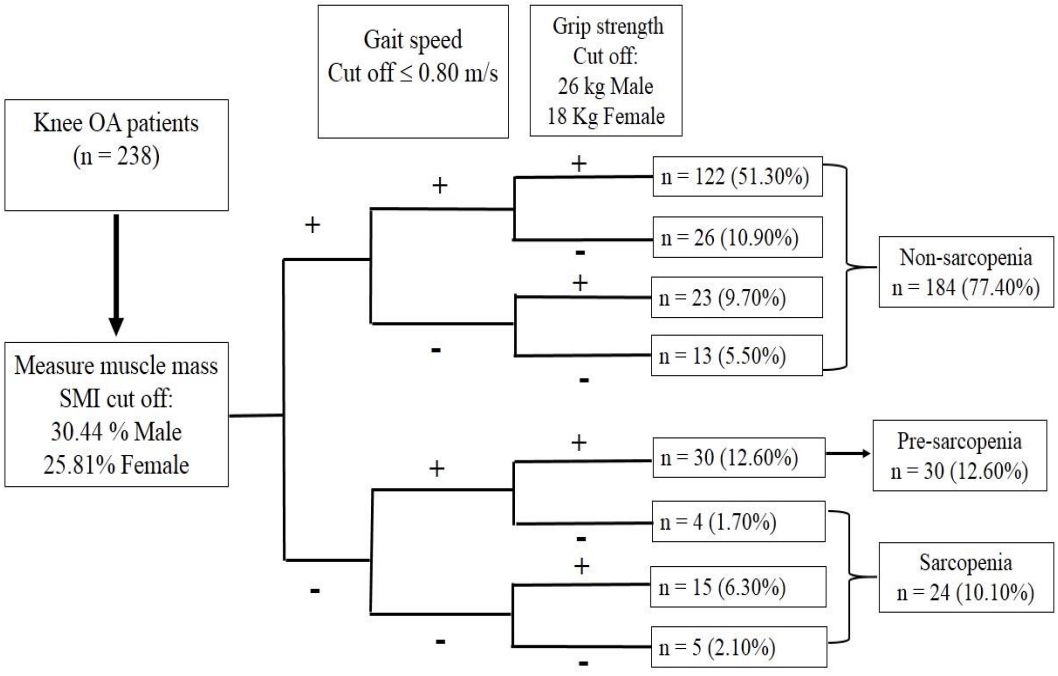


Figure 1 The prevalence of sarcopenia in knee OA patients (n=238).

2. The characteristic of participants

We were categorized knee OA patients into 3 groups, sarcopenia, pre-sarcopenia and non-sarcopenia. The demographic data of three groups as shown in **Table 1.** Mean of age in OA patients with pre-sarcopenia was younger than those of sarcopenia and non-sarcopenia (p = 0.004)**.** BMI, percentage of fat mass, fat mass, and visceral fat rating were higher in OA patients with sarcopenia and pre-sarcopenia**.**

Table 1 The demographic data of knee OA patients with sarcopenia, pre-sarcopenia and non-sarcopenia

	Knee OA patients			
	Non-sarcopenia	**Pre-sarcopenia**	**Sarcopenia**	***p***
	n = 184	**n = 30**	**n = 24**	
		Mean ± SEM		
Gender (F/M)	162/22	29/1	24/0	
Age (years)	65.86±0.51	60.93±1.43	66.96±1.18 [b]	0.001
Body composition				
BMI (kg/m^2)	24.35±0.26	29.13±0.69	29.42±0.62 [a]	<0.001
Percentage of fat mass (%)	32.92±0.44	43.13±0.81	44.24±0.77 [a]	<0.001
Fat mass (kg)	20.02±0.49	31.35±1.34	31.16±1.21 [a]	<0.001
Visceral fat rating	8.68±0.28	12.26±0.47	13.00±0.43 [a]	<0.001
ASM (kg)	17.73±0.31	17.34±0.37	16.50±0.33	0.33
ASMI (kg/m^2)	7.23±0.09	7.00±0.13	6.97±.121	0.41
SMI (%)	29.78±0.24	24.14±0.22	23.79±0.30 [a]	<0.001

F: female, M: male, BMI: Body mass index, ASM: Appendicular skeletal muscle mass, ASMI: Appendicular skeletal muscle mass index, and SMI: Skeletal muscle index

[a] $p < 0.05$ sarcopenia versus non-sarcopenia, by analysis of variance with post hoc analysis

[b] $p < 0.05$ sarcopenia versus pre-sarcopenia, by analysis of variance with post hoc analysis

3. Self-report pain, health-related quality of life and physical activity in knee OA patients with sarcopenia

Self-report pain, especially, VAS was statistically different in sarcopenia ($p < 0.05$) but WOMAC score was not different between groups. PCS score of SF-12 in sarcopenia was significantly lower than that in non-sarcopenia ($p < 0.05$). The mean value of physical activity in sarcopenia was lowest among three groups indicating that sarcopenia in knee OA patients had sedentary lifestyle ($p < 0.05$) as shown in **Table 2.**

Table 2 VAS, WOMAC, SF-12 and PAQ-EJ in knee OA patients with sarcopenia

	Knee OA patients			
	Non-sarcopenia	**Pre-sarcopenia**	**Sarcopenia**	***p***
	n = 184	**n = 30**	**n = 24**	
		Mean ± SEM		
VAS (0-10)	3.84±0.17	4.17±0.49	5.50±0.51 [a]	0.008
WOMAC				
Pain (0-10)	2.47±0.15	2.91±0.38	3.09±0.51	0.28
Stiffness (0-10)	2.60±0.17	3.30±0.51	2.54±0.57	0.35
Physical disability (0-10)	2.89±0.15	3.37±0.40	3.61±0.47	0.19
Total score (0-10)	2.66±0.14	3.20±0.39	3.08±0.46	0.29
SF-12				
PCS (0-100)	39.30±0.66	35.67±1.58	33.61±1.68 [a]	0.004
MCS (0-100)	49.66±0.68	48.17±1.61	51.57±1.87	0.40
Physical activity				
PAQ-EJ (MET hours/week)	55.65±3.26	39.76±6.70	21.7533±7.66 [a]	0.01

VAS: Visual analogue scale, WOMAC: Western Ontario and McMaster Universities Osteoarthritis Index, SF-12: 12-Item short form health survey, PCS: Physical health composite scores, MCS: Mental health composite scores, PAQ-EJ: physical activity questionnaire for elderly Japanese in Thai version and MET: metabolic equivalent of task.

[a] $p < 0.05$ sarcopenia versus non-sarcopenia, by analysis of variance with post hoc analysis

4. Frequency of falls in knee OA patients with sarcopenia

According to frequency of falls in the past 6 months, 37.50% of sarcopenia group had at least 1 fall in the past 6 months, 30.00% in pre-sarcopenia, and 24.50% in non-sarcopenia group. There were not different in fall rates among three groups ($p > 0.05$) as shown in **Table 3**. .

The odds ratio (OR) of fall rates for sarcopenia compared with non-sarcopenia were 0.54. However, there was no difference in fall risk between groups ($p = 0.17$).

Table 3 Frequency of falls in the past 6 months in knee OA patients with sarcopenia

	Knee OA patients			
	Non-sarcopenia	**Pre-sarcopenia**	**Sarcopenia**	***p***
	n = 184	**n = 30**	**n = 24**	
Frequency of falls in 6 months				
No (%)	75.50	70.00	62.50	
Yes (%)	24.50	30.00	37.50	0.35

5. Metabolic syndrome (MetS) in knee OA patients with sarcopenia

The mean value of HOMA-IR levels in sarcopenia group had significantly higher than pre-sarcopenia and non-sarcopenia group ($p < 0.05$). MetS components, knee OA patients with sarcopenia and pre-sarcopenia had significantly higher in waist circumference ($p < 0.001$) but fasting glucose, lipid profile and blood pressure were no differences among three groups **(Table 4).**

The prevalence rates of MetS, these were 66.66% in sarcopenia, 46.66% in pre-sarcopenia and 39.13% in non-sarcopenia group. There was significantly higher MetS rates in sarcopenia compared with non-sarcopenia group ($p = 0.003$).

In risk factors of MetS for sarcopenia, odds ratio (OR) was 15.11 ($p < 0.001$), suggesting that the effect of metabolic risk factors raised the risk of sarcopenia in OA patients.

Table 4 Metabolic syndrome (MetS) in knee OA patients with sarcopenia

	Knee OA patients			
	Non-sarcopenia	**Pre-sarcopenia**	**Sarcopenia**	***p***
	n = 184	**n = 30**	**n = 24**	
	Mean ± SEM			
Metabolic risk factors				
Insulin (μU/ml)	5.1397±0.03	5.73±0.82	7.54±0.99	0.10
HOMA-IR	1.32±0.11	1.31±0.20	2.16±0.34 [b]	0.04
LDL (mg/dl)	130.37±2.61	155.73±5.05	134.79±7.2	0.08
MetS components				
Waist circumference (cm)	85.40±0.70	94.80±1.30	96.20±1.58 [a]	<0.001
Fasting glucose (mg/dl)	98.69±1.61	97.46±2.34	106.26±4.47	0.23
HDL cholesterol (mg/dl)	56.12±1.02	52.48±1.89	54.08±3.19	0.36
Triglyceride (mg/dl)	117.71±3.95	122.20±9.08	132.91±9.63	0.40
SBP (mmHg)	130.86±0.69	129.10±1.22	131.45±1.66	0.55
DBP (mmHg)	79.99±0.73	78.83±2.05	80.33±1.44	0.81
Number of component of MetS				
≤1 (%)	37.50	16.68	4.18	
2 (%)	23.37	36.66	29.16	
≥3: MetS (%)	39.13	46.66	66.66 [a]	0.003¶

HOMA-IR: Homeostatic model assessment of insulin resistance, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, SBP: Systolic blood pressure and DBP: Diastolic blood pressure

[a] $p < 0.05$ sarcopenia versus non-sarcopenia, by analysis of variance with post hoc analysis

[b] $p < 0.05$ sarcopenia versus pre-sarcopenia and non-sarcopenia, by analysis of variance with post hoc analysis

6. Vitamin D, calcium and phosphorus homeostasis in knee OA patients with sarcopenia

There were not different in levels of vitamin D, calcium and phosphorus homeostasis among vitamin D deficiency, insufficiency and sufficiency groups ($p > 0.05$) as shown in **Table 6**.

The percentage number of knee OA patients who was normal vitamin D status among vitamin D deficiency, insufficiency and sufficiency groups as presented in **Figure 2.**

Table 6 Vitamin D, calcium and phosphorus homeostasis in knee OA patients with sarcopenia

	Knee OA patients			
	Non-sarcopenia n = 184	Pre-sarcopenia n = 30	Sarcopenia n = 24	*p*
	Mean ± SEM			
25 (OH)D (ng/ml)	23.62±0.64	21.74±1.13	24.98±1.63	0.35
Albumin (mg/dl)	4.29±0.01	4.26±0.03	4.30±0.04	0.67
Calcium (mg/dl)	9.20±0.05	9.34±0.06	9.37±.073	0.28
Phosphorus (mg/dl)	3.65±0.03	3.51±0.10	3.56±0.07	0.24
PTH (pg/ml)	49.27±1.76	55.38±3.95	50.66±5.18	0.44

25(OH)D: 25-hydroxyvitamin D

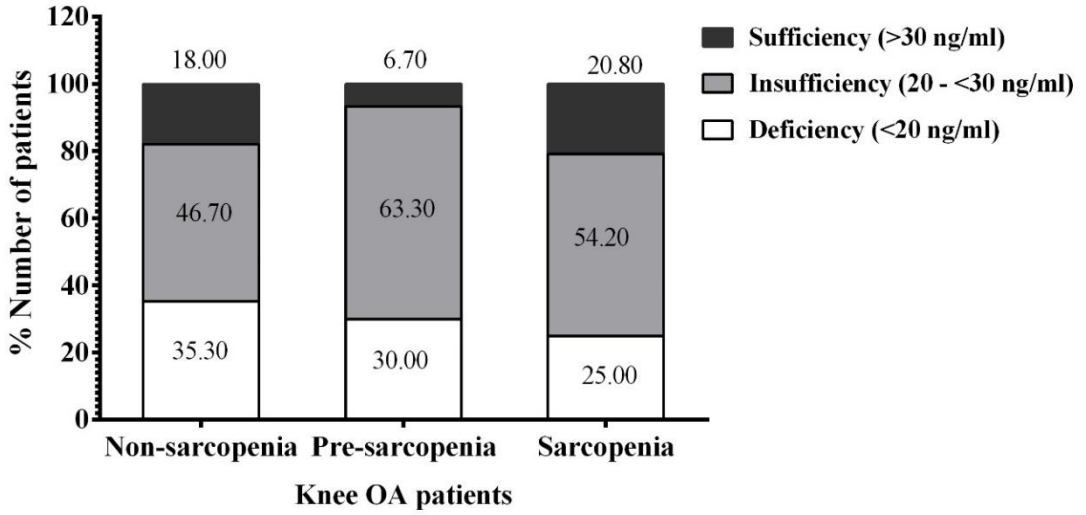


Figure 2. The percentage number of knee OA patients who was vitamin D sufficiency

7. Inflammation, adipokine and oxidative stress

Levels of hs-CRP and leptin in knee OA patients with sarcopenia were higher than obesity and non-sarcopenia group ($p < 0.001$) as shown in **Table 6.**

Table 6 Levels of inflammation, adipokine and oxidative stress in knee OA patients with vitamin D deficiency

	Knee OA patients			
	Non-sarcopenia n = 184	Pre-sarcopenia n = 30	Sarcopenia n = 24	*p*
	Mean ± SEM			
hs-CRP (mg/dl)	1.51±0.15	2.18±0.30	4.89±1.15 [a]	<0.001
IL-6 (pg/ml)	12.81±0.81	15.65±2.10	9.20±1.60	0.08
Leptin (ng/ml)	20.87±1.34	41.43±3.91	39.79±4.86 [a]	<0.001
Protein carbonyl (nmol/mg)	0.60±0.03	0.70±0.13	0.87±0.19	0.08

hs-CRP: high-sensitivity C-reactive protein and IL-6: interleukin-6

[a] $p < 0.05$ sarcopenia versus pre-sarcopenia and non-sarcopenia, by analysis of variance with post hoc

8. Muscle strength and physical performance in knee OA patients with sarcopenia

Muscle strength, as follows, grip strength and knee extension force were not different among three groups ($p > 0.05$), whereas, physical performance (gait speed, TUGT, STS and 6MWT) were lower in sarcopenia than in pre-sarcopenia and non-sarcopenia group ($p < 0.01$) as shown in **Table 7**.

Table 7 Muscle strength and physical performance in knee OA patients with sarcopenia

	Knee OA patients			
	Non-sarcopenia	**Pre-sarcopenia**	**Sarcopenia**	***p***
	n = 184	**n = 30**	**n = 24**	
	Mean ± SEM			
Muscle strength				
Grip strength:				
Dominant (kg)	22.45±0.42	22.78±0.64	19.96±1.07	0.10
Non dominant (kg)	20.43±0.42	20.45±0.80	18.49±1.07	0.26
Knee extension force:				
Symptomatic Leg (N)	369.21±6.69	392.80±19.03	368.09±21.07	0.43
Non-symptomatic Leg (N)	393.45±6.56	412.50±14.48	367.20±15.15	0.16
Physical performances				
Gait speed (m/s)	0.98±0.01	1.00±0.03	0.72±.030 [b]	<0.001
TUGT (s)	9.63±0.17	9.64±0.30	11.92±0.71 [b]	<0.001
STS (s)	14.51±0.33	15.52±0.79	17.75±0.95 [a]	0.004
6MWT (m)	379.04±5.93	375.13±10.95	299.00±18.08 [b]	<0.001

TUGT: Timed up and go test, STS: sit to stand, and 6MWT: Six-Minute Walk test.

[a] $p < 0.05$ sarcopenia versus non-sarcopenia, by analysis of variance with post hoc analysis

[b] $p < 0.05$ sarcopenia versus pre-sarcopenia and non-sarcopenia, by analysis of variance with post

APPENDIX B

Self-report assessments and physical performance tests

วันที่.....ครั้งที่ทดสอบ.....	เลขที่.....

แบบบันทึกข้อมูลส่วนบุคคล

อายุ.....ปี เพศ ☐ ชาย ☐ หญิง

ความดันโลหิต.....mmHg ส่วนสูง.....cm. น้ำหนัก.....Kg.

โปรดทำเครื่องหมาย ✓ ลงใน ❑ ที่ตรงกับความเป็นจริงและเติมข้อความในช่องว่าง

- ได้รับการผ่าตัดข้อเข่า หรือเปลี่ยนข้อเข่าเทียม ❑ ใช่ ❑ ไม่ใช่
- เป็นโรคข้ออักเสบรูมาตอยด์ ข้ออักเสบจากการติดเชื้อ ❑ ใช่ ❑ ไม่ใช่
- เป็นโรคตับ ❑ ใช่ ❑ ไม่ใช่
- เป็นโรคไต ❑ ใช่ ❑ ไม่ใช่
- เป็นโรคหลอดเลือดหัวใจโดยมีอาการเจ็บ แน่นบริเวณหน้าอก หรือใส่อุปกรณ์เครื่องกระตุ้นหัวใจ (pacemaker) ❑ ใช่ ❑ ไม่ใช่
- ขณะนี้ท่านได้รับประทานวิตามินดีเป็นอาหารเสริม ❑ ใช่ ❑ ไม่ใช่

หากตอบ ใช่ ระยะเวลาที่ท่านทานวิตามินดี.....ปี.....เดือน

ท่านจงตอบแบบสอบถามตามข้อต่อไปนี้ หากไม่ท่านเข้าใจข้อความใด โปรดซักถามเจ้าหน้าที่

1. ท่านมีโรคประจำตัวใดบ้าง โปรดทำเครื่องหมาย ✓ หรือ X ในช่องว่าง

❑ ไม่มีโรคประจำตัว ❑ โรคความดันโลหิตสูง
❑ ไขมันในเลือดสูง ❑ โรคอัมพฤกษ์/อัมพาต
❑ โรคหัวใจและหลอดเลือด ❑ โรคไต
❑ โรคเบาหวาน ❑ โรคตับ
❑ โรคระบบทางเดินหายใจหรือปอด ❑ โรคอื่นๆ เช่น.....

2. รอบ 6 เดือนที่ผ่านมา ท่านเคยหกล้มหรือไม่

❑ ไม่เคย ❑ เคย (จำนวนครั้ง).....

หากท่านมีประวัติเคยล้ม ท่านเกิดการบาดเจ็บหรือไม่

❑ ไม่มี ❑ มี อาการบาดเจ็บอย่างไร ?.....

3. ท่านเริ่มเป็นโรคข้อเข่าเสื่อมตั้งแต่ อายุ.....ปี

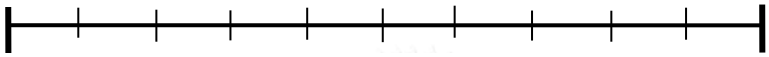
4. ท่านมีอาการปวดข้อเข่าข้างใด

   ❑ ข้างขวา ❑ ข้างซ้าย

   ❑ ปวดเท่ากันทั้งสองข้าง

   ❑ ปวดทั้งสองข้าง โดยมีอาการปวดข้าง ☐ ขวา ☐ ซ้าย มากที่สุด

5. ท่านจงทำเครื่องหมาย กากบาท (X) ลงบนเส้นตรง เพื่อระบุระดับอาการปวดเข่าของท่าน โดยจุดเริ่มต้นไม่มีอาการปวดเลย จากนั้นระดับอาการปวดค่อยๆ รุนแรง เพิ่มมากขึ้น ตามยาวของเส้นตรง จนถึงจุดสิ้นสุดมีอาการปวดมากที่สุด



ไม่มีปวดเลย ปวดมากที่สุด

พลิกหน้าถัดไป เพื่อตอบคำถามเกี่ยวอาการปวดเข่า กิจวัตรประจำวันในการทำงาน และคุณภาพชีวิตของท่าน

สำหรับนักกายภาพบำบัด หรือเจ้าหน้าที่

- ความผิดปกติของรูปร่างข้อเข่า

  ❑ ไม่มี ❑ Varus ❑ Valgus ❑ อื่นๆ

.....

- ความผิดปกติของโครงสร้างกล้ามเนื้อ กระดูก

.....

.....

.....

แบบสอบถาม Modified WOMAC ฉบับภาษาไทย

แบบสอบถามmodified WOMAC ฉบับภาษาไทย เป็นการประเมินอาการของผู้ป่วยโรคข้อเสื่อม ประกอบด้วยคำถาม 3 สวน คือ คำถามระดับความปวด ระดับอาการข้อฝืด และระดับความสามารถในการใช้งานขอ โปรดกรุณา X กาเครื่องหมายหรือ ○ วงกลมลอมรอบตัวเลขให้ตรงกับอาการของท่านมากที่สุด

ระดับความปวด (0 – 10) โดย 0 หมายถึงไมปวดเลย และ 10 หมายถึง ปวดมากจนทนไมได้

1. ปวดขณะเดิน

0 1 2 3 4 5 6 7 8 9 10

ไม่ปวดเลย ปวดมากจนทนไม่ได้

2. ปวดขณะขึ้นลงบันได

0 1 2 3 4 5 6 7 8 9 10

ไม่ปวดเลย ปวดมากจนทนไม่ได้

3. ปวดข้อตอนกลางคืน

0 1 2 3 4 5 6 7 8 9 10

ไม่ปวดเลย ปวดมากจนทนไม่ได้

4. ปวดข้อขณะอยู่เฉยๆ

0 1 2 3 4 5 6 7 8 9 10

ไม่ปวดเลย ปวดมากจนทนไม่ได้

5. ปวดข้อขณะยืนลงน้ำหนัก (ขาข้างนั้นรับน้ำหนักตัว)

0 1 2 3 4 5 6 7 8 9 10

ไม่ปวดเลย ปวดมากจนทนไม่ได้

ระดับอาการข้อฝืด,ขอยึด (0–10) โดย 0 หมายถึง ไม่มีอาการฝืดเลย และ 10 หมายถึง มีอาการฝืดมากที่สุด

1. ข้อฝืดช่วงเช้า (ขณะตื่นนอน)

0 1 2 3 4 5 6 7 8 9 10

ไม่ปวดเลย ปวดมากจนทนไม่ได้

2. ข้อฝืดในช่วงระหว่างวัน

0 1 2 3 4 5 6 7 8 9 10

ไม่ปวดเลย ปวดมากจนทนไม่ได้

ระดับความสามารถในการใช้งานขอ (0 – 10)
โดย 0 หมายถึง ทำได้ดีมาก และ 10 หมายถึง ไมสามารถทำกิจกรรมนั้นๆ ได้

1. การลงบันได

0 1 2 3 4 5 6 7 8 9 10

ไม่ปวดเลย ปวดมากจนทนไม่ได้

2. การขึ้นบันได

0 1 2 3 4 5 6 7 8 9 10

ไม่ปวดเลย ปวดมากจนทนไม่ได้

3. การลุกขึ้นยืนจากท่านั่ง

0 1 2 3 4 5 6 7 8 9 10

ไม่ปวดเลย ปวดมากจนทนไม่ได้

4. การยืน

0 1 2 3 4 5 6 7 8 9 10

ไม่ปวดเลย ปวดมากจนทนไม่ได้

5. การเดินบนพื้นราบ

0 1 2 3 4 5 6 7 8 9 10

ไม่ปวดเลย ปวดมากจนทนไม่ได้

6. การขึ้นลงรถยนต์

0 1 2 3 4 5 6 7 8 9 10

ไม่ปวดเลย ปวดมากจนทนไม่ได้

7. การไปซื้อของนอกบ้านหรือการไปจ่ายตลาด

0 1 2 3 4 5 6 7 8 9 10

ไม่ปวดเลย ปวดมากจนทนไม่ได้

8. การใส่กางเกง (ในท่ายืน)

0 1 2 3 4 5 6 7 8 9 10

ไม่ปวดเลย ปวดมากจนทนไม่ได้

9. การลุกจากเตียง

0 1 2 3 4 5 6 7 8 9 10

ไม่ปวดเลย ปวดมากจนทนไม่ได้

10. การถอดกางเกง (ในท่ายืน)

0 1 2 3 4 5 6 7 8 9 10

ไม่ปวดเลย ปวดมากจนทนไม่ได้

11. การเข้าออกจากห้องอาบน้ำ

0 1 2 3 4 5 6 7 8 9 10

ไม่ปวดเลย ปวดมากจนทนไม่ได้

12. การนั่ง (ยืนลงนั่งเก้าอี้)

0 1 2 3 4 5 6 7 8 9 10

ไม่ปวดเลย ปวดมากจนทนไม่ได้

13. การเข้า-ออกจากส้วม (การนั่งลง-ลุกขึ้นจากโถส้วม)

0 1 2 3 4 5 6 7 8 9 10

ไม่ปวดเลย ปวดมากจนทนไม่ได้

14. การทำงานบ้านหนักๆ (ล้างห้องน้ำ รดน้ำต้นไม้ในสวน ถูบ้าน ตัดหญ้า หิ้วของหนัก)

0 1 2 3 4 5 6 7 8 9 10

ไม่ปวดเลย ปวดมากจนทนไม่ได้

15. การทำงานบ้านเบาๆ (ทำกับข้าว ล้างถ้วยจาน ปัดฝุ่น เช็ดโต๊ะ)

0 1 2 3 4 5 6 7 8 9 10

ไม่ปวดเลย ปวดมากจนทนไม่ได้

สำหรับนักกายภาพบำบัด หรือเจ้าหน้าที่

Pain_____

Stiffness_____

Physical disability_____

คะแนนรวมทั้งหมด : _____

แบบสอบถาม SF-12 สำหรับประเมินสุขภาพ

คำแนะนำการตอบแบบสอบถาม

กรุณาตอบแบบสอบถามให้ครบทุกข้อ โปรดอ่านและตอบคำถามแต่ละข้อให้ถูกต้องตามความเป็นจริงโดยขีดเครื่องหมายถูกในช่องสี่เหลี่ยม ที่ท่านเห็นว่าตรงกับลักษณะของท่านมากที่สุด

1. ในภาพรวม ท่านคิดว่าสุขภาพของท่าน

ดีเยี่ยม	ดีมาก	ดี	ปานกลาง	เลว
❑	❑	❑	❑	❑

ท่านคิดว่า สุขภาพของท่านเป็นปัญหา/อุปสรรคในการทำกิจกรรมของท่านหรือไม ถ้าใช่ มากน้อยแคไหน

2. กิจกรรมที่ใชกำลังปานกลาง เชน การยกโตะ การทำความสะอาดปดกวาด เช็ดถูบาน หรือหิ้วของ กลับจากตลาด

- ❑ ใช่ เป็นปัญหา/อุปสรรคอย่างมาก
- ❑ ใช่ เป็นปัญหา/อุปสรรคเล็กน้อย
- ❑ ไม่ เป็นปัญหา/อุปสรรคเลย

3. การเดินขึ้นตึก 2-3 ชั้น หรือเดินขึ้นเนิน

- ❑ ใช่ เป็นปัญหา/อุปสรรคอย่างมาก
- ❑ ใช่ เป็นปัญหา/อุปสรรคเล็กน้อย
- ❑ ไม่ เป็นปัญหา/อุปสรรคเลย

ในช่วง 1 เดือนที่ผ่านมา ท่านมีปัญหาการทำงานหรือทำกิจวัตรประจำวัน ซึ่งเป็นผลเนื่องมาจากสุขภาพร่างกายของท่านหรือไม่

4. ทำงานได้ปริมาณน้อยลงกว่าที่ต้องการ

- ❑ ไม่ใช่
- ❑ ใช่

5. ไม่สามารถทำงานได้ทุกอย่างตามที่ตั้งใจไว้ ต้องเลือกทำบางอย่างเท่านั้น

- ❑ ไม่ใช่
- ❑ ใช่

ในช่วง 1 เดือนที่ผ่านมา ท่านประสบปัญหาเกี่ยวกับการทำงานหรือทำกิจวัตรประจำวัน ซึ่งเป็นผลเนื่องมาจากปัญหาเกี่ยวกับอารมณ์หรือจิตใจของท่าน เช่น รู้สึกซึมเศร้า หรือ วิตกกังวล หรือไม่ ?

6. ทำงานได้ปริมาณน้อยลงกว่าที่ต้องการ

❑ ไม่ใช่

❑ ใช่

7. ทำงานหรือทำกิจกรรมอื่นๆ โดยปราศจากความระมัดระวัง สับเพร่า เลินเล่อ อย่างที่เคย

❑ ไม่ใช่

❑ ใช่

8. ในช่วง 1 เดือนที่ผ่านมา ปัญหาการเจ็บปวดตามร่างกาย ทำให้ท่านไม่สามารถทำงานประจำวันได้ตามปกติ (งานในบ้านและนอกบ้าน) มากน้อยเพียงใด?

ไม่เลย	เล็กน้อย	ปานกลาง	ค่อนข้างมาก	มากที่สุด
❑	❑	❑	❑	❑

คำถามต่อไปนี้เกี่ยวข้องกับอารมณ์ความรู้สึกที่เกิดขึ้นกับท่านในช่วง 1 เดือนที่ผ่านมา กรุณาให้คำตอบที่ตรงกับความรู้สึกของท่านมากที่สุด ในแต่ละคำถามเกิดขึ้นบ่อยเพียงใดในช่วง 1 เดือนที่ผ่านมา ?

9. ท่านรู้สึกใจสงบ ใจนิ่ง มีสมาธิ

ตลอดเวลา	เกือบตลอดเวลา	ค่อนข้างบ่อย	บางครั้ง	นานๆ ครั้ง	ไม่เลย
❑	❑	❑	❑	❑	❑

10. ท่านรู้สึกแข็งแรง กระปรี้กระเปร่า สดชื่น

ตลอดเวลา	เกือบตลอดเวลา	ค่อนข้างบ่อย	บางครั้ง	นานๆ ครั้ง	ไม่เลย
❑	❑	❑	❑	❑	❑

11. ท่านรู้สึกเศรา หดหู่ ไม่ร่าเริง

ตลอดเวลา	เกือบตลอดเวลา	ค่อนข้างบ่อย	บางครั้ง	นานๆ ครั้ง	ไม่เลย
❑	❑	❑	❑	❑	❑

12. ในช่วง 1 เดือนที่ผ่านมา ปัญหาเกี่ยวกับสุขภาพหรืออารมณ์ของท่าน มีผลรบกวนต่อเวลาการมีกิจกรรมทางสังคมของท่าน เช่น ไปเยี่ยมเพื่อน หรือ ญาติหรือไม่?

ตลอดเวลา	เกือบตลอดเวลา	บางครั้ง	นานๆ ครั้ง	ไม่มีเลย
❑	❑	❑	❑	❑

แบบสอบถามการเคลื่อนไหวร่างกายสำหรับผู้สูงอายุ

คำชี้แจง: ต่อไปนี้เป็นแบบสอบถามเกี่ยวกับกิจกรรมต่างๆ ที่ท่านได้ทำไปแล้วภายในช่วง 7 วันที่ผ่านมา โปรดคิดถึงกิจกรรมการเคลื่อนไหวร่างกายต่างๆ ที่ท่านปฏิบัติ เช่น ขณะทำงานบ้านและงานสนามของบ้านท่านเอง การเดินทางไป-กลับระหว่างสถานที่ต่างๆ และการใช้เวลาว่างในการทำกิจกรรมเพื่อพักผ่อนหย่อนใจ การออกกำลังกายหรือเล่นกีฬา

1. ในช่วง 7 วันที่ผ่านมา ท่านได้เดินหรือปั่นจักรยานไปทำธุระ เช่น ไปร้านค้า ตลาด ห้างสรรพสินค้า หรือพาเด็กๆ ไปโรงเรียน หรือเดินไปทำธุระนอกบ้าน [การเดินทาง] (<u>หากตอบ</u> ไม่เคย กรุณาข้ามไปข้อถัดไป)

❑ ไม่เคย ❑ นาน ๆ ครั้ง (1-2 วัน)

❑ บ้างครั้ง (3-4 วัน) ❑ เป็นประจำ (5-7 วัน)

ในแต่ละวันที่ท่านตอบนั้น ท่านใช้ระยะเวลาในการทำกิจกรรมเท่าใด

❑ น้อยกว่า 30 นาที ❑ 30 นาทีขึ้นไป – 1 ชั่วโมง

❑ 1 ชั่วโมงขึ้นไป – 2 ชั่วโมง ❑ มากกว่า 2 ชั่วโมงขึ้นไป

2. ในช่วง 7 วันที่ผ่านมา ท่านได้เคลื่อนไหวร่างกาย<u>ระดับเบา</u>หรือทำกิจกรรมสันทนาการต่างๆ เช่น เดินเล่นยามว่าง เล่นกีฬาแบบยืดกล้ามเนื้อ เล่นกอล์ฟ โยนบอลลิ่ง เล่นเปตอง

(<u>หากตอบ</u> ไม่เคย กรุณาข้ามไปข้อถัดไป)

❑ ไม่เคย ❑ นาน ๆ ครั้ง (1-2 วัน)

❑ บ้างครั้ง (3-4 วัน) ❑ เป็นประจำ (5-7 วัน)

ในแต่ละวันที่ท่านตอบนั้น ท่านใช้ระยะเวลาในการทำกิจกรรมเท่าใด

❑ น้อยกว่า 1 ชั่วโมง ❑ 1 ชั่วโมงขึ้นไป – 2 ชั่วโมง

❑ 2 ชั่วโมงขึ้นไป – 4 ชั่วโมง ❑ มากกว่า 4 ชั่วโมงขึ้นไป

3. ในช่วง 7 วันที่ผ่านมา ท่านได้เคลื่อนไหวร่างกาย<u>ระดับปานกลางหรือค่อนข้างหนัก</u> เช่น เดินเร็วเพื่อออกกำลังกาย วิ่งเหยาะๆ เต้นรำหรือ ลีลาศ เล่นเทนนิส ว่ายน้ำ ไท้ฉี โยคะ (<u>หากตอบ</u> ไม่เคย กรุณาข้ามไปข้อถัดไป)

❑ ไม่เคย ❑ นาน ๆ ครั้ง (1-2 วัน)

❑ บ้างครั้ง (3-4 วัน) ❑ เป็นประจำ (5-7 วัน)

ในแต่ละวันที่ท่านตอบนั้น ท่านใช้ระยะเวลาในการทำกิจกรรมเท่าใด

❑ น้อยกว่า 30 นาที ❑ 30 นาทีขึ้นไป – 1 ชั่วโมง

❑ 1 ชั่วโมงขึ้นไป – 2 ชั่วโมง ❑ มากกว่า 2 ชั่วโมงขึ้นไป

4. ในช่วง 7 วันที่ผ่านมา ท่านได้ออกกำลังกายแบบให้แรงต้านเพื่อเพิ่มความแข็งแรงของกล้ามเนื้อ เช่น ยกน้ำหนัก การใช้อุปกรณ์ออกกำลังกายที่มีแรงต้าน เช่น ยางยืด ตุ้มยกน้ำหนัก เครื่องออกกำลังกาย อุปกรณ์ออกกำลังกายตามสวนสาธารณะหรือสวนสุขภาพ (หากตอบ ไม่เคย กรุณาข้ามไปข้อถัดไป)

❑ ไม่เคย ❑ นาน ๆ ครั้ง (1-2 วัน)

❑ บ้างครั้ง (3-4 วัน) ❑ เป็นประจำ (5-7 วัน)

ในแต่ละวันที่ท่านตอบนั้น ท่านใช้ระยะเวลาในการทำกิจกรรมเท่าใด

❑ น้อยกว่า 30 นาที ❑ 30 นาทีขึ้นไป – 1 ชั่วโมง

❑ 1 ชั่วโมงขึ้นไป – 2 ชั่วโมง ❑ มากกว่า 2 ชั่วโมงขึ้นไป

5. ในช่วง 7 วันที่ผ่านมา ท่านได้ทำกิจกรรมงานบ้านระดับเบา เช่น กวาดบ้าน ดูดฝุ่น ทำอาหาร ล้างจาน ซักผ้า และ รดน้ำต้นไม้ เป็นต้น (หากตอบ ไม่เคย กรุณาข้ามไปข้อถัดไป)

❑ ไม่เคย ❑ นาน ๆ ครั้ง (1-2 วัน)

❑ บ้างครั้ง (3-4 วัน) ❑ เป็นประจำ (5-7 วัน)

ในแต่ละวันที่ท่านตอบนั้น ท่านใช้ระยะเวลาในการทำกิจกรรมเท่าใด

❑ น้อยกว่า 1 ชั่วโมง ❑ 1 ชั่วโมงขึ้นไป – 2 ชั่วโมง

❑ 2 ชั่วโมงขึ้นไป – 4 ชั่วโมง ❑ มากกว่า 4 ชั่วโมงขึ้นไป

6. ในช่วง 7 วันที่ผ่านมา ท่านได้ทำกิจกรรมงานบ้านระดับปานกลางหรือค่อนข้างหนัก เช่น ทำความสะอาดหน้าต่าง ขัดพื้น ทำงานสวน เช่น กวาดใบไม้ ตัดแต่งกิ่งไม้ขนาดเล็ก ถอนหญ้าด้วยมือ งานซ่อมแซมบ้าน เช่น ทาสี เป็นต้น (หากตอบ ไม่เคย กรุณาข้ามไปข้อถัดไป)

❑ ไม่เคย ❑ นาน ๆ ครั้ง (1-2 วัน)

❑ บ้างครั้ง (3-4 วัน) ❑ เป็นประจำ (5-7 วัน)

ในแต่ละวันที่ท่านตอบนั้น ท่านใช้ระยะเวลาในการทำกิจกรรมเท่าใด

❑ น้อยกว่า 1 ชั่วโมง ❑ 1 ชั่วโมงขึ้นไป – 2 ชั่วโมง

❑ 2 ชั่วโมงขึ้นไป – 4 ชั่วโมง ❑ มากกว่า 4 ชั่วโมงขึ้นไป

7. ในช่วง 7 วันที่ผ่านมา ท่านได้ทำกิจกรรมหรือทำงานที่มีการเคลื่อนไหว<u>ออกแรงมาก</u> เช่น ยกหรือแบกหามของหนัก ขุดดินโดยใช้จอบหรือเสียม (<u>หากตอบ</u> ไม่เคย กรุณาข้ามไป)

❑ ไม่เคย ❑ นาน ๆ ครั้ง (1-2 วัน)

❑ บ้างครั้ง (3-4 วัน) ❑ เป็นประจำ (5-7 วัน)

ในแต่ละวันที่ท่านตอบนั้น ท่านใช้ระยะเวลาในการทำกิจกรรมเท่าใด

❑ น้อยกว่า 1 ชั่วโมง ❑ 1 ชั่วโมงขึ้นไป – 2 ชั่วโมง

❑ 2 ชั่วโมงขึ้นไป – 4 ชั่วโมง ❑ มากกว่า 4 ชั่วโมงขึ้นไป

ขอบคุณที่ท่านให้ความร่วมมือในการตอบแบบสอบถามนี้

สำหรับนักกายภาพบำบัด หรือเจ้าหน้าที่ PAQ-EJ score (MET hr/week) = number of days x time x intensity weight	
intensity weight	
- transportation	= 2.8 x ..... x ..... =.....MET hr/week
- light exercise/sports	= 3 x ..... x ..... =.....MET hr/week
- moderate exercise/sports	= 4.3 x .....x ..... =.....MET hr/week
- resistance exercise/sports	= 3 x ..... x ..... =.....MET hr/week
- light housework	= 2 x ..... x ..... =.....MET hr/week
- moderate housework	= 2.5 x ..... x ..... =.....MET hr/week
- labor	= 2.8 x ..... x ..... =.....MET hr/week
	TOTAL =.....MET hr/week

การทดสอบสมรรถภาพร่างกาย

ส่วนสูง.....น้ำหนัก.....BMI.....

Waist circumference.....cm.

1. Hand grip (dominant hand)

	มือขวา (kg)	มือซ้าย (kg)
ครั้งที่ 1		
ครั้งที่ 2		
ครั้งที่ 3		

ค่าปกติ ผู้ชาย ≥ 26 kg ผู้หญิง ≥ 18 kg

แปลผล ❑ ปกติ ❑ แรงบีบมือต่ำ

2. Quadriceps strength (lb)

	ขาขวา	ขาซ้าย
ครั้งที่ 1		
ครั้งที่ 2		
ครั้งที่ 3		

แปลผล ❑ ปกติ ❑ แรงบีบมือต่ำ

3. The usual 4-meter gait speed

gait speed	เวลา (s)	m/s
ครั้งที่ 1		
ครั้งที่ 2		

ค่าปกติ ระยะเวลา > 0.8 m/s

แปลผล ❑ ปกติ ❑ เดินช้า

4. Time up to go test (3 เมตร)

TUG	เวลา (s)
ครั้งที่ 1	
ครั้งที่ 2	

ค่าปกติ ระยะเวลา <10 s

แปลผล ❑ ปกติ ❑ ความสามารถในการทรงตัวต่ำ

5. Sit to stand test (5 ครั้ง)

	เวลา (s)
ครั้งที่ 1	
ครั้งที่ 2	

ค่าปกติ ระยะเวลา <12 s

แปลผล ❑ ปกติ ❑ เคลื่อนไหวช้า

6. The Six-Minute Walk (6MWT) test

25 X _____รอบ _____

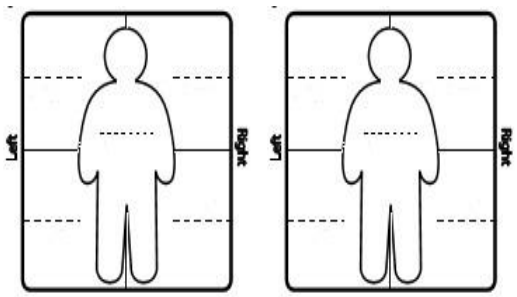
ระยะทาง.....

ค่าปกติ ระยะทาง 400 m

แปลผล ❑ ปกติ ❑ สมรรถภาพต่ำ

8. Bioelectrical Impedance Analysis (BIA)

Segmental Lean Segmental Fat



SMI (%) =ASM/body weight X 100

= .....%

ค่าปกติ ผู้ชาย ≥ 30.44% ผู้หญิง ≥ 25.81%

แปลผล ❑ ปกติ ❑ มวลกล้ามเนื้อต่ำ

Fat % .....

Fat Mass.....

Visceral fat rating.....

VITA

Miss Pacharee Manoy was born on 10th July 1979 in Phatthalung, Thailand. She graduated with Bachelor's Degree of Science (Physical Therapy) from Chiang Mai University in 2001 and graduated with Master's Degree of Science (Sports Medicine) from Chulalongkorn University in 2007. She is a lecturer at Department of Physical Therapy, School of Allied Health Science, University of Phayao.

Miss Pacharee presented her poster presentation at Joint conference in Medical Sciences 2015 in Bangkok, Thailand and Osteoarthritis Research Society International (OARSI) World Congress on Osteoarthritis 2016 in Amsterdam, Netherlands and the meeting abstract was published in Osteoarthritis and Cartilage 24 (2016) S80. Additionally, she received a Best Travel Award from OARSI.

Publication

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