2012 年度　修士論文

健康成人における血中ビタミンD濃度と体力との関連

The Relationship of Vitamin D with Cardiorespiratory Fitness and Muscular Strength in Japanese Adults

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5011A051-0

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Chapter 1: Background & Purpose

1-1 Vitamin D

Vitamin D is a group of fat-soluble vitamin. And it plays not only an important role in maintaining metabolism and growth of bone by responsible for intestinal absorption of calcium and phosphate, but also accumulating evidence indicates that it has a protective role against many diseases, including cancer, cardiovascular diseases, diabetes, autoimmune diseases, and infectious diseases.[1-3] Human gets vitamin D largely from exposure to sunlight which induces vitamin D production in the skin, and also a few from diet. However, causes of variety of factors, such as limited to geographical latitude, season and supplement use, vitamin D deficiency is spread in the world.[1,4,5]

1-1-1 Sources and Metabolism of Vitamin D

Vitamin D was got from exposure to sunlight, from their diet, and from dietary supplements.[1] Solar ultraviolet B radiation (wavelength, 290 to 315 nm) penetrates the skin and converts 7-dehydrocholesterol to previtamin D_3 (Fig.1-1).[1] And few foods naturally contain or are fortified with vitamin D_2 or D_3 (Fig 1-1).[1] Vitamin D_2 comes from plant sources, such as mushrooms, and is converted from ergosterol found in fungi or yeast on exposure to UVB light. Similarly, vitamin D_3 comes from animal sources, such as fatty fish, and is converted from 7-dehydro -cholesterol found in human skin on exposure to UVB light.[6] Both are used in over-the-counter vitamin D supplements, but the form available by prescription in the United States is vitamin D_2.[1]

Vitamin D from the skin and diet is metabolized in the liver to 25-hydroxyvitamin D(25(OH)D); 25(OH)D is metabolized in the kidneys by the enzyme 25-hydroxyvitamin D-1α-1 hydroxylase (CYP27B1) to active form of vitamin D, 1,25-dihydroxyvitamin D (1,25(OH)2D)(Fig.1-1).[1] The renal production of 1,25(OH)2D is tightly regulated by plasma parathyroid hormone (PTH) levels and serum calcium and phosphorus levels(Fig.1-1).[1]

1-1-2 Mechanism and Effect of Vitamin D

1,25(OH)2D which active form of vitamin D is released into the circulation following the final converting step in the kidney, and is transported to various target organs by binding to vitamin D-binding protein (VDBP) which a carrier protein in the plasma. Then, 1,25(OH)2D mediates its effect by binding to the vitamin D receptor (VDR), which is principally located in the nuclei of target cells. The binding of 1,25(OH)2D to the VDR allows the VDR to act as a transcription factor that modulates the gene expression of transport proteins such as calcitonin which are involved in calcium absorption in the intestine.[7]

Without vitamin D, only 10 to 15% of dietary calcium and about 60% of phosphorus is absorbed (Fig 1-1).[8-10] Therefore, 1,25(OH)2D can affect primarily to maintain metabolism and growth bone

via VDR activation. It is to maintain skeletal calcium balance by promoting calcium absorption in the intestines, promoting bone resorption by increasing osteoclast number, maintaining calcium and phosphate levels for bone formation, and allowing proper functioning of PTH to maintain serum calciumLevels.[11-15)] PTH enhances the resorption of calcium and stimulates the kidneys to produce 1,25(OH)2D. [8-10)]

The potential effect of vitamin D on skeletal muscle structure and function is receiving a great deal of attention on the basis of both clinical and basic science research.[16)] Experimental studies have revealed VDR in skeletal muscle, and vitamin D have been found affect muscle metabolism by stimulating de novo protein synthesis, increasing the proportion of type II muscle fibers and improving muscle function.[17)] Vitamin D, VDR and optimal muscle health are directly linked, however, they are raising many questions that need to be investigated.

Vitamin D is also effect on other organs, including brain, heart, skin, and immune system, via VDRs which are expressed by cells.[11,18-19)]

1-1-3 Definition of Vitamin D Deficiency

Vitamin D level is assessed by 25 (OH)D level. And vitamin D deficiency is defined by most experts as 25 (OH)D level of less than 20 ng per milliliter (50 nmol per liter).[12-13, 20-21)] 25(OH)D levels are inversely associated with PTH levels until the former reach 30 to 40 ng per milliliter (75 to 100 nmol per liter), at which point PTH levels begin to level off.[13-15)] Furthermore, intestinal calcium transport increased by 45 to 65% in women when 25(OH)D levels were increased from an average of 20 to 32 ng per milliliter (50 to 80 nmol per liter).[22)]

Then, given such data, a level of 25(OH)D of 21 to 29 ng per milliliter (52 to 72 nmol per liter) can be considered to indicate a relative insufficiency of vitamin D, and a level of 30 ng per milliliter or greater can be considered to indicate sufficient vitamin D.[23)] Vitamin D intoxication is observed when serum 25(OH)D level is greater than 150 ng per milliliter (374 nmol per liter). [23)]

1-1-4 Causes of Vitamin D Deficiency

There are many causes of vitamin D deficiency, including reduced skin synthesis (such as season, latitude, sunscreen use and skin pigment) and absorption of vitamin D from food and heritable disorders of vitamin D metabolism and responsiveness.[1)] (Table 1-1)

1-1-5 Consequences of Vitamin D Deficiency

As we know, vitamin D deficiency causes osteomalacia and low serum vitamin D levels have been associated with falls and low bone mineral density.[24)] Some studies have shown that supplementation with vitamin D and calcium may improve bone mineral density slightly, and decrease the risk of falls and fractures , specifically in older than 65 years.[24-25)] Vitamin D deficiency also causes muscle

weakness which associated with falls.[12,20] Skeletal muscles have a vitamin D receptor and may require vitamin D for maximum function .[12]

Also, evidence for effect of vitamin D deficiency is associated with chronic diseases including cancer,[26] cardiovascular disease,[27-28] autoimmune diseases, osteoarthritis and diabetes. The reason is that vitamin D deficiency increased insulin resistance, decreased insulin production, and was associated with the metabolic syndrome.[29] And, vitamin D deficiency is associated with congestive heart failure and blood levels of inflammatory factors, including C-reactive protein and interleukin-10.[30, 31]

1-1-6 Prevalence of Vitamin D Deficiency

Despite the importance of vitamin D sufficiency is well known, with the use of the definitions of vitamin D deficiency, it has been estimated that 1 billion people worldwide have vitamin D deficiency or insufficiency.[12-15, 20-21] According to several studies, 40 to 100% of U.S. and European elderly men and women still living in the community (not in nursing homes) are deficient in vitamin D. [12-15, 20-21] Some studies show that, at the end of the winter, 42% of 15 to 49 years old black girls and women throughout the United States had 25-hydroxyvitamin D levels below 20 ng per milliliter, and 32% of healthy students, physicians, and residents at a Boston hospital were found to be vitamin D deficient, despite drinking a glass of milk and taking a multivitamin daily and eating salmon at least once week.[1] In other studies in Saudi Arabia, the United Arab Emirates, Australia, Turkey, India, and Lebanon, 30 to 50% of children and adults had 25-hydroxyvitamin D levels under 20 ng per milliliter. [1]

In Japan, there were few studies about vitamin D status. A study has reported that vitamin D deficiency is common in Japanese adults who working in office during seasons with limited sunlight in Northeast Kyushu Japan (33.4-33.5 ℃N).[32] Other study has reported that vitamin D deficiency and vitamin D deficiency rickets has been prevalence in Hokkaido (45 ℃N), which has a higher latitude than other regions and low hours of sunlight in Japan.[33] However, data collection from youth and male was scant in Japan, and the range of subjects' age was small. Therefore, in this study, we investigated vitamin D status both female and male with larger range of age in Japan.

1-2 Physical Fitness

Physical fitness has been defined as a set of attributes or characteristics that people have or achieve that relates to the ability to perform physical activity. It comprises two related concepts: performance-related fitness which is a task-oriented definition based on the ability to perform specific aspects of sports or occupations and health-related fitness which is a state of health and well-being. In these, health-related fitness is linked to fitness components that may lower risks such as metabolic syndrome, and it is also considered a measure of the body's ability to function efficiently and

effectively in work and leisure activities, to be healthy, to resist hypokinetic diseases, and to meet emergency situations.[34] Thus, it can be used to estimate the healthy status in common.

1-2-1 Health-related Fitness and Vitamin D

Health-related fitness is to improve the overall health of the individual by gaining and maintaining a desirable level of fitness through sound physical activity and exercise habits. The four components of physical fitness are cardiorespiratory fitness, muscular endurance and strength, body composition, and flexibility.[34] In these components, cardiorespiratory fitness, which is the ability of the heart and lungs to keep your muscles supplied with oxygen for long periods of continuous activity and exercise, was unusually used as method. And, muscular strength, which is the ability of a muscle or group of muscles to exert maximum force in a single contraction, was frequently used as method. Therefore, in this study, we selected cardiorespiratory fitness and muscular strength as health-related fitness.

Moreover, there were many studies that showed vitamin D was associated with physical fitness, but the mechanisms underlying this associated are unclear. Maybe it could be considered that vitamin D is to maintain skeletal muscle health via VDR activation which has been identified on skeletal muscle.

1-2-2 Cardiorespiratory Fitness and Vitamin D

Cardiorespiratory fitness refers to the ability of the circulatory and respiratory systems to supply oxygen to skeletal muscles during sustained physical activity. Exercise makes these systems more efficient by enlarging the heart muscle, enabling more blood to be pumped with each stroke, and increasing the number of small arteries in trained skeletal muscles, which supply more blood to working muscle. Several studies were proved that it was benefited to reduce the risk of heart disease, lung cancer, type 2 diabetes, stroke, and other disease.[35] In addition, the higher levels of cardiorespiratory fitness are independently and inversely associated with rates of all-cause and cardiovascular mortality.[36]

Vitamin D deficiency is independently associated with all-cause and cardiovascular mortality via its role in muscle strength, function.[37-39] Animal studies have demonstrated that vitamin D supplementation improved cardiac morphology.[40] Because cardiorespiratory fitness is primarily a function of maximal cardiac output and maximal arteriovenous O_2 difference (an index of muscle function), vitamin D insufficiency may also have a significant effect on cardiorespiratory fitness.[41] In few human studies, higher serum 25(OH)D levels were positively associated with better cardiorespiratory fitness.[41-44]

1-2-3 Muscular Strength and Vitamin D

Muscular strength is generally defined as the ability to exert maximal force using maximum or near

maximum resistance during limited repetitions, and it typically is developed using resistance training. By this type of training, muscular strength can be simulated to increase on various physiological levels. And also, it can be an indicator of overall health or a measure of progress during resistance or rehabilitation training programs. Recent data, muscular strength was evidenced to be inversely associated with metabolic syndrome incidence.[45)]

Until recently, several observational studies in older adults showed a positive association between vitamin D status and muscular strength.[46-49)] The association between vitamin D status and muscle strength and performance may not be unique to older individuals. A positive relationship between 25(OH)D levels and muscle power, force, velocity, and jump height was noted in 99 postmenarchal girls aged 12-14 years with low 25(OH)D levels (mean 21.3 nmol/L).[50)] And some studies in adults were shown that vitamin D status was associated with muscular strength,[51)] but some studies were just shown that the relationship was existed in hand not leg.[52)]

1-3 Purpose

According to these results, we knew that vitamin D deficiency was associated with physical fitness. But abundant of these studies were in the old, and few studies were in the young, and many of them were in Westerners, and few of them in Japanese. However, vitamin D and physical fitness were influenced by race and environment. Thus, we cannot know that the relationship of vitamin D with physical fitness existing in Westerners is also existed in Japanese.

Moreover, several studies reported that the aging process led to decreased receptor expression in skeletal muscle cells in women.[53)] And another was shown that 11 elderly patients were treated with a vitamin D analog (1,25(OH)2D) and calcium supplements, resulting in an increase in the number of fast-twitch type IIa muscle fibers and a decrease in the number of type IIb muscle fibers.[54)] Therefore, we consider that serum 25(OH)D level may have been related to physical fitness, and hypothesize that the relationship is also existed in Japanese, though physical fitness in Japanese is different from in Westerners.

From the above, in the present study, the first aim was to investigate vitamin D status in Japanese. And the second aim was to ascertain the association between vitamin D level and physical fitness, including cardiorespiratory fitness and muscular strength, in Japanese adults.

Figure 1-1 The metabolism of vitamin D

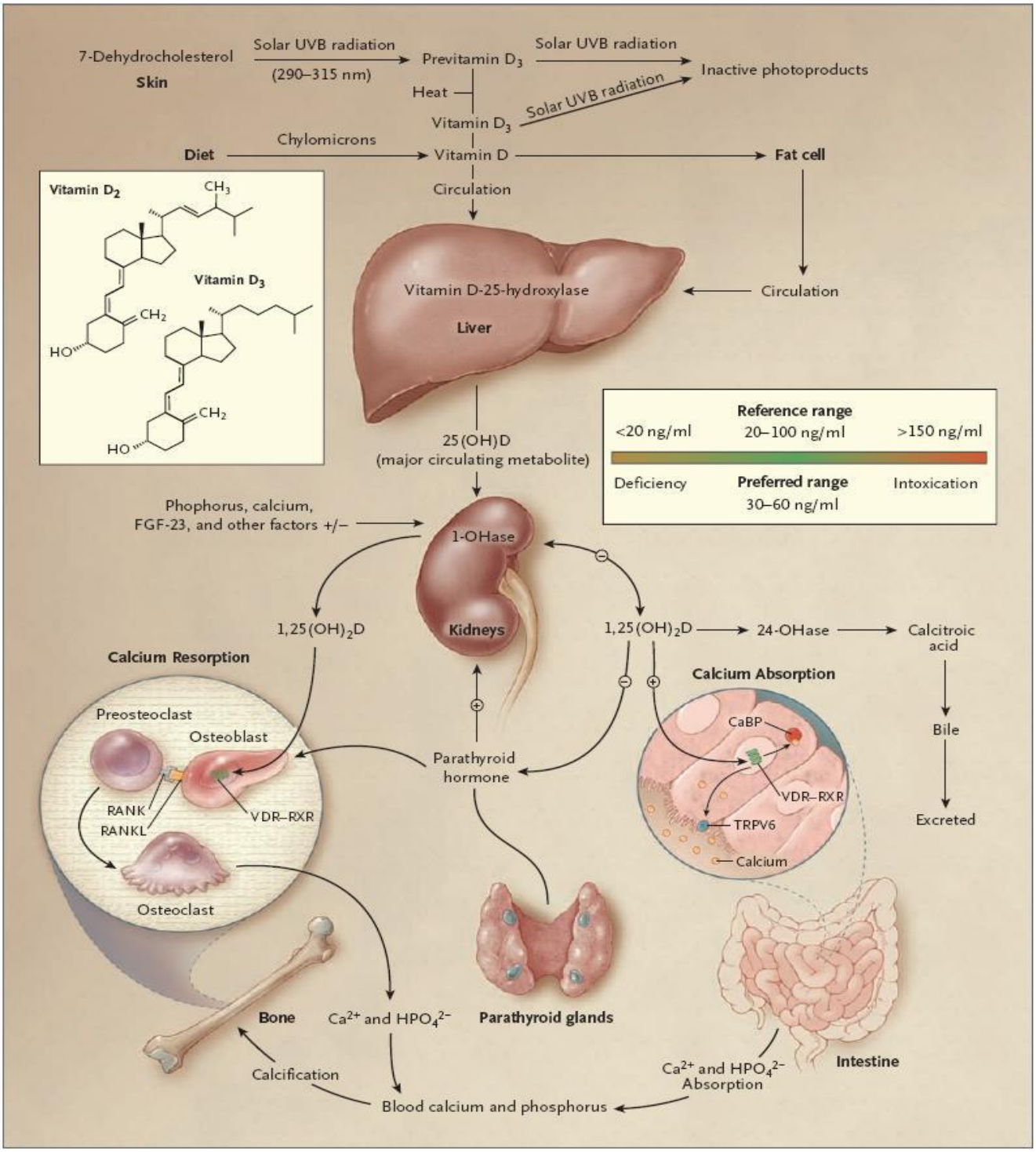

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Table 1-1 Causes of vitamin D deficiency[1)]

Cause	Effect
Reduced skin synthesis	
Sunscreen use - absorption of UVB radiation by sunscreen	Reduces vitamin D_3 synthesis - SPF 8 by 92.5%, SPF 15 by 99%
Skin pigment - absorption of UVB radiation by melanin	Reduces vitamin D_3 synthesis by as much as 99%
Aging - reduction of 7-dehydrocholesterol in the skin	Reduces vitamin D_3 synthesis by about 75% in a 70-year-old
Season, latitude, and time of day - number of solar UVB photons reaching the earth depending on zenith angle of the sun (the more oblique the angle, the fewer UVB photons reach the earth)	Above about 35 degrees north latitude (Atlanta), little or no bitamin D_3 can be produced from November to February
Patients with skin grafts for burns - marked reduction of 7-dehy-drocholesterol in the skin	Decreases the amount of vitamin D_3 the skin can produce
Decreased bioavailability	
Malabsorption - reduction in fat absorption, resulting from cystic fibrosis, celiac disease, Whipple's disease, Crohn's disease, bypass surgery, medications that reduce cholesterol absorption, and other causes	Impairs the body's ability to absorb vitamin D
Obesity - sequestration of vitamin d in body fat	Reduces availability of vitamin D
Increased catabolism	
Anticonvulsants, glucocorticoids, HAART(AIDS treatment), and antirejection medications - bining to the steroid and xenobiotic receptor or the pregnane X receptor	Activates the destruction of 25-hydr- oxyvitamin D and 1,25-dihydroxyvitamin D to inactive calcitroic acid
Breast-feeding	
Poor vitamin D content in human milk	Increases infant risk of vitamin D deficiency when breast milk is sole source of nutrition

Table 1-1 (Continued.) [1)]

Cause	Effect
Decreased synthesis of 25-hydroxyvitamin D	
Liver failure	
Mild-to-moderate dysfunction	Causes malabsorption of vitamin D, but production of 25-hydroxyvitamin D is possible
Dysfunction of 90% or more	Results in inability to make sufficient 25-hydroxyvitamin D
Increased urinary loss of 25-hydroxyvitamin D	
Nephrotic syndrome - loss of 25-hydroxyvitamin D bound to vitamin D-binding protein in urine	Results in substantial loss of 25-hydroxybitamin D to urine
Decreased synthesis of 1,25-dihydroxyvitamin D	
Chronic kidney disease	
Stages 2 and 3 (estimated glomerular filtration rate, 31 to 89 mL/min/1.73 m^2)	
Hyperphosphatemia increases fibroblast growth factor 23, which decreases 25-hydroxyvitamin D-1α-hydroxylase activity	Causes decreased fractional excretion of phosphorus and decreased serumLevels of 1,25-dihydroxyvitamin D
Stages 4 and 5 (estimated glomerular filtration rate <30 mL/min/1.73 m^2)	
Inability to produce adequate amounts of 1,25-dihydroxyvitamin D	Causes hypocalcemia, secondary hyper -parathyro -idism, and renal bone disease
Heritable disorders - rickets	
Pseudovitamin D deficiency rickets (vitamin D - dependent rickets type 1) - mutation of the renal 25-hydroxyvitamin D-1α-hydroxylase gene (CYP27B1)	Causes reduced or no renal synthesis of 1,25-dihydroxyvitamin D
Vitamin D - resistant rickets (vitamin D - dependent rickets type 2) - mutation of the vitamin D receptor gene	Causes partial or complete resistance to 1,25-dihydroxyvitamin D action, resulting in elevated levels of 1,25-dihydroxyvitamin D

Table 1-1 (Continued.) [1)]

Cause	Effect
Heritable disorders - rickets	
Vitmain D - dependent rickets type 3 - overproduction of hormoneresponsive-element binding proteins	Prevents the action of 1,25-dihydroxyvitamin D in transcription, causing target-cell resistance and elevated levels of 1,25-dihydroxyvitamin D
Autosomal dominant hypophosphatemic rickets - mutation of the gene for fibroblast growth factor 23, preventing or reducing its breakdown	Causes phosphaturia, decreased intestinal absorption of phosphorus, hypophos -phatemia, and decreased renal 25-hydroxyvitamin D-1α-hydroxylase activity, resulting in low-normal or low levels of 1,25-dihydroxyvitamin D
X-linked hypophosphatemic rickets - mutation of the PHEX gene, leading to elevated levels of fibroblast growth factor 23 and other phosphatonins	Causes phosphaturia, decreased intestinal absorption of phosphorus, hypophosphate -mia, and decreased renal 25-hydroxyvita -min D-1α-hydroxylase activity, resulting in low-normal or low levels of 1,25-dihydroxy -vitamin D
Acquired disorders	
Tumor-induced osteomalacia - tumor secretion of fibroblast growth factor 23 and possibly other phosphatonins	Causes phosphaturia, decreased intestinal absorption of phosphorus, hypophosp -hatemia, and decreased renal 25-hydroxy –vitaminD-1α-hydroxylase activity, resulting in low-normal or low levels of 1,25-dihydroxy -vitamin D
Primary hyperparathyroidism - increase in levels of parathyroid hormone, causing increased metabolism of 25-hydroxyvitamin D to 1,25-hydroxyvitamin D	Decreases 25-hydroxyvitamin D levels and increases 1,25-dihydroxyvitamin D levels that are high-normal or elevated

Table 1-1 (Continued.) [1)]

Cause	Effect
Acquired disorders	
Granulomatous disorders, sarocidosis, tuberculo -sis, and other conditions, including some lympho -mas - conversion by macrophages of 25-hydroxy -vitmain D to 1,25-dihydroxyvitamin D	Decreases 25-hydroxyvitamin D levels and increases 1,25-dihydroxyvitamin D levels
Hyperthyroidism - enhanced metabolism of 25-hy -droxyvitamin D	Reduces levels of 25-hydroxyvitamin D

Chapter 2: Methods

Subjects

Data for this cross-sectional study were obtained from 95 healthy men and women living in Saitama, Japan (35 ℃N latitude) by internet, poster and advertisement of newspaper. The population consisted of 34 men and 61 women, from 20 to 69 years of age. Participants were generally healthy and free of medications and had no contraindications to any of the study procedures. Participants were excluded if the used any medications or had any chronic medical conditions that might affect study results. Participants were also excluded if they had cardiovascular disease, diabetic, hypertension, osteoporosis injuries by osteoporosis or had a history of them. Participants were also excluded if they were taking a daily dietary-supplement during the previous year. Data was collected from April to May 2012. Informed consent and assent were obtained from all participants and the study protocol was approved by the Ethical committee of Waseda University.

Body composition

Body composition was measured by using various techniques according to standard procedures. Standing height with minimal clothing and with the shoes off was measured to the nearest 0.1cm (YL-65, Yagami, Inc, Nagoya, Japan). Body weight was measured to the nearest 0.1kg by using an electronic body composition scale (Inner Scan BC-660, Tanita Co, Itabashi Tokyo, Japan). The proportion of body fat were measured by using dual-energy x-ray absorptiometry(DXA)(Hologic QDT-4500, DXA Scanner, Hologic Inc. Whaltham, MA, USA). The body mass index (BMI) was calculated by dividing body weight in kilograms by the square of height in meters (kg/m^2). Waist circumference was measured at the umbilical region by using a nonelastic measuring tape.

Clinical Chemistries

Venous blood samples were collected in the morning after fasting for at least 12-h from April to May 2012. The serum samples were stored at -80 ° until analyzed. Serum 25(OH) D concentrations was determined at the first baseline visit using an enzyme-linked immunosorbent assay (immundiagnostik AG, Bensheim Germany) with intra- and inter- kit of 8.9% and 10.6%.

PTH can increase the metabolism of 25(OH)D to 1,25(OH)2D as vitamin D deficiency progresses. As serum 25(OH)D concentration was low, serum PTH level would increase. So low levels of 25(OH)D and high levels of serum PTH are considered to represent vitamin D deficiency.[55)] Serum PTH was also determined at the first baseline visit using an enzyme-linked immunosorbent assay (immune-diagnostik AG, Bensheim, Germany) with inter assay 3.5% and intra 5.2%.

Cardiorespiratory Fitness

Cardiorespiratory fitness was measured by a maximal graded exercise test on a cycle

ergometer(Monark Exercise AB, Varberg, Sweden) and quantified as maximal oxygen uptake ($\dot{V}O_2$ max). The initial work load was adjusted to 30-60W, and the work rate was increased thereafter by 15 W/min until the subject could not maintain the required pedaling frequency of 60 rpm.[56] Heart rate(HR) (Life Scope BSM-1101, NIHON KODEN Corp., Tokyo, Japan) and a rating of perceived exertion (RPE) were monitored throughout the exercise. During the progressive exercise test, the expired gas of subjects was collected and the rates of oxygen consumption(VO_2) and carbon dioxide production(VCO_2) were measured and averaged over 30-s intervals using an automated breath-by-breath gas analyzing system (Aeromonitor AE-280S, Minato Medical Science, Tokyo, Japan) . During the latter stages of the test, each subject was verbally encouraged by the test operators to give a maximal effort. The highest observed value of VO_2 during the exercise test was considered to be the maximal oxygen uptake (mL/kg/min), and achievement of $\dot{V}O_2$max was accepted if at least two of the following four criteria were achieved: the $\dot{V}O_2$ curve showed a leveling off, subject's maximal HR was >95% the age-predicted maximal HR (220-age), a respiratory exchange ratio in excess of 1.0 and the subject achieved an RPE of 19 or 20.

Muscle Strength

Hand grip strength of both hands was measured using a hand grip dynamometer (YX, YAGAMI Inc., Nagoya, Japan) in units of kilograms. Test was taken place 4 trails and 2 for each hand alternately. Subjects held the dynamometer with the arm completely extended in standing position and exert the maximal force with each contraction trail. The average value for 4 trails was used for statistical analysis.

Leg extension power was measured using a leg extension power measurement system (Anaero Press 3500, Combi Wellness, Tokyo, Japan). Subject sat on the seat and placed both feet on the footplate. The waist and ankles of subject were firmLy fastened by Velcro straps. The seat was moved to a position at which the knee joint angle was about 90 degrees. The load of the footplate was set to equal the subject's weight. Subjects were instructed to push the footplate by extending both legs as quickly and powerfully as possible. Leg extension power was measured five times with rest periods of 15 seconds between trials. The highest recorded outputs was taken as the definitive measurement.

Physical Activity

Physical activity was measured by the Kenz Lifecorde (LC; SUZUKEN Co Ltd, Nagoya, Japan) which is a recent addition to the growing number of uniaxial accelerometer options; it offers comparable instrument outputs with several potentially attractive features for researchers and practitioners.[57] The subjects were taught how to use the instrument, and were told to wear it on their belt or waistband at the right midline of the thigh from the moment they got up until they went to bed except while bathing or swimming, for seven consecutive days. The activity monitor was firmLy

attached to their clothes at the waist by a clip.[57] Data from days 1 and 7 were discarded because a full day of information was not available for those days. Daily and total movement counts per day were converted to minutes per day spent in moderate (3-6METs) and vigorous (>6METs) physical activity by the software for the device.[58]

Statistical analysis

Measured and calculated values are presented as means ± SD. Differences among the means for gender were examined by using T-test for all independent variables unless PTH and MVPA. PTH and MVPA were not normally distributed; therefore, they were examined by using Manu-Whitney U-test. We divided that >10 ng/mL was considerably deficiency;10~20 ng/mL was deficiency; and subjects who were vitamin D sufficiency were only 3 person in this study, so we categorized insufficiency and sufficiency as third group(20ng/mL≤). Gender differences of the distribution of serum 25(OH)D concentration were tested by using X^2 test. Pearson's product correlations were calculated between the independent variables (PTH, $\dot{V}O_2$max, grip strength, leg extension power) and serum 25(OH)D concentration. Multiple linear regression analysis was used to identify the significant association of serum 25(OH) D with $\dot{V}O_2$max, grip strength and leg extension power. In these former analyses, measure variables which were not normally distributed were severally transformed. This transformation yielded normal distributions, and the transformed variables were used. Comparison of grip strength and leg extension power of subjects in three serum 25(OH)D concentration categories by using Kruskal Wallis test. All statistical tests used a significance level of 5%. Statistical analyses were performed by using SPSS 20.0 for Windows (SPSS Japan Inc., Tokyo, Japan).

Chapter 3: Results

3-1 Vitamin D Status in Japan

Participant Characteristics

A total of 95 adults 20 to 69 years of age (34 male, 61 female) participated in this cross-sectional study. BMI, percent body fat, grip strength, leg extension power and $\dot{V}O_2$max were significantly higher in the male than in the female ($p<0.05$). And there were no significant gender differences in MVPA and PTH (Table 3-1, $p>0.05$).

Prevalence of Vitamin D Status

According to the definition of vitamin D deficiency (less than 20ng/L), [1)] 78% of subjects were vitamin D deficiency, and merely 3% of them were sufficiency. The prevalence of vitamin D deficiency (84%) among women was higher than among men (65%)(Figure 3-1). Moreover, serum 25(OH)D concentration in female was significantly lower than in male(Table 3-1, $p=0.029$).

In this study, the subjects were divided into three groups by serum 25(OH)D concentration. Then, there were no significant gender differences in the distribution of vitamin D status (Table 3-1, $p>0.05$).

Serum 25(OH)D Concentration and Serum PTH

There were no significant relationships between serum 25(OH)D and PTH in male($r=-0.086$, $p=0.627$) and in female ($r=-0.150$,$p=0.248$). We just merely found a significant trend between serum 25(OH)D and PTH in all participants($r=-0.184$, $p=0.074$) (Figure 3-2).

3-2 Relationship of Vitamin D with Cardiorespiratory Fitness

In the present study, 86 subjects met $\dot{V}O_2$max criteria. Therefore, we used data of 86 subjects to analyze. There was a significant relationship between serum 25(OH)D concentration and $\dot{V}O_2$max in all participants($r=0.224$, $p=0.041$) (Figure 3-3). And the results from multiple linear regression analysis examining the associated of serum 25(OH)D with $\dot{V}O_2$max were shown that serum 25(OH)D was positively related to significant $\dot{V}O_2$max after adjusted by gender, age($\beta=0.184$,$p=0.039$) and there was a borderline statistically significant relationship after further adjusted by BMI ($\beta=0.165$, $p=0.054$). But this relationship was disappeared after further adjustment for physical activity ($\beta=0.159$, $p=0.074$) (Table 3-2).

3-3 Relationship of Vitamin D with Muscular Strength

Relationship between Vitamin D and Hand Grip Strength

Hand grip strength was not normally distributed, so we transformed it to X^{-1} before performing the analysis. Though it was shown significant negative correlation between serum 25(OH)D concentration

and X^{-1}, in fact, there was significant positive correlation between serum 25(OH)D concentration and hand grip strength(r=-0.258,p=0.012) (Figure 3-4 (A)). In addition, the relationship remained after adjusted by gender, age (β=-0.137,p=0.044), BMI(β=-0.148,p=0.027), moderate to vigorous physical activity(β=-0.168, p=0.016) and $\dot{V}O_2$max(β=-0.149,p=0.034) (Table 3-3).

We also observed a significant positive trend for hand grip strength level across incremental vitamin D categories (Figure 3-5, p=0.026).

Relationship Vitamin D and Leg Extension Power

There was significant positive correlation between serum 25(OH)D concentration and leg extension power (r=0.251,p=0.014) (Figure 3-4 (B)). And the relationship remained after adjusted by gender, age (β=0.140, p=0.041), BMI(β=-0.154,p=0.020), moderate to vigorous physical activity(β=0.188, p=0.006) and $\dot{V}O_2$max(β=0.140,p=0.027) (Table 3-3).

As like the results about grip strength, we also observed a significant positive trend for hand grip strength level across incremental vitamin D categories. (Figure 3-6, p=0.017).

Table 3-1 Subject characteristics

n=95	male (n=34)	female (n=61)	P Value
Age(years)	42.0±16.3	44.4±12.8	0.563
Height(cm)	170.3±5.9	158.1±4.9	<0.001
Weight(kg)	69.0±6.4	53.0±7.3	<0.001
BMI(kg/m^2)	23.9±2.6	21.2±2.8	<0.001
Body Fat(%)	17.9±4.6	26.5±5.0	<0.001
Serum 25(OH)D (ng/mL)	16.7±9.6	12.5±6.9	0.029
Distribution of serum 25(OH)D			0.074
>10 ng/mL(%)	26.5	44.3	
10~20 ng/mL(%)	38.2	39.3	
20≤ng/mL(%)	35.3	16.4	
Hand Grip strength (kg)	43.0±8.2	25.6±3.9	<0.001
Leg extension power (kg/w)	1523.5±444.0	694.6±232.0	<0.001
$\dot{V}O_2$max(mL/kg/min)	38.1±8.4	30.7±6.3	<0.001
MVPA(min/day)	33.5±19.9	33.8±22.0	0.759
PTH(pg/mL)	58.5±21.3	66.0±2.8	0.100

Data presented as mean ± SD. PTH, Parathyroid hormone. VO_2max, Maximal oxygen uptake. MVPA, Moderate to vigorous physical activity.

P value: for gender difference.

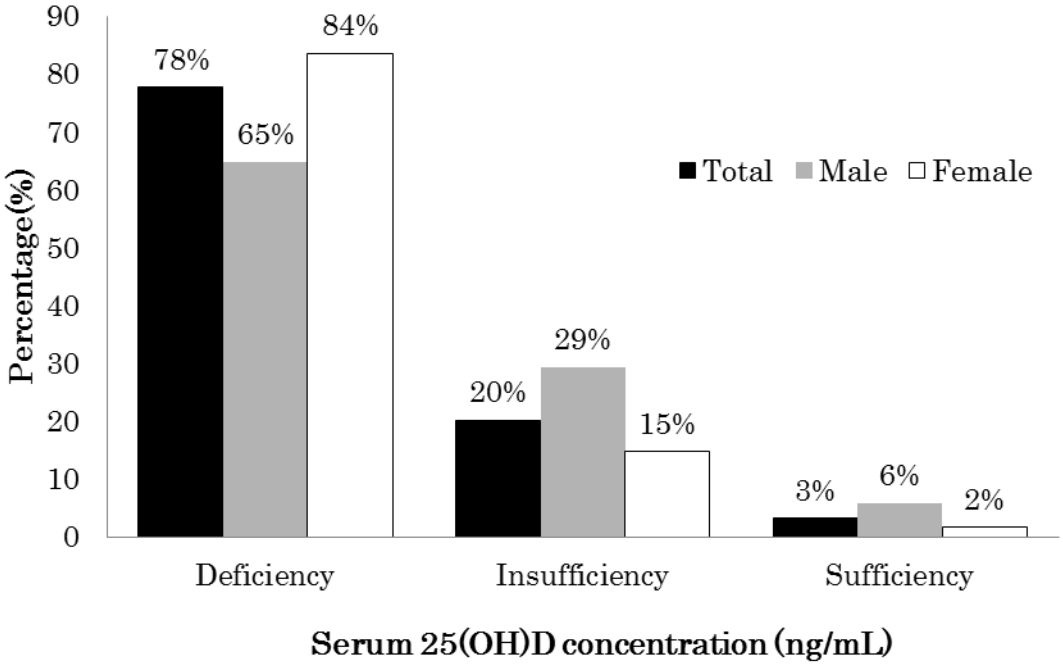

Figure 3-1

High prevalence of vitamin D insufficiency and deficiency in healthy Japanese adults.

Serum 25(OH)D concentration: ＜20ng/mL was deficiency; 20~29.9ng/mL was insufficiency;≧30ng/mL was sufficiency.

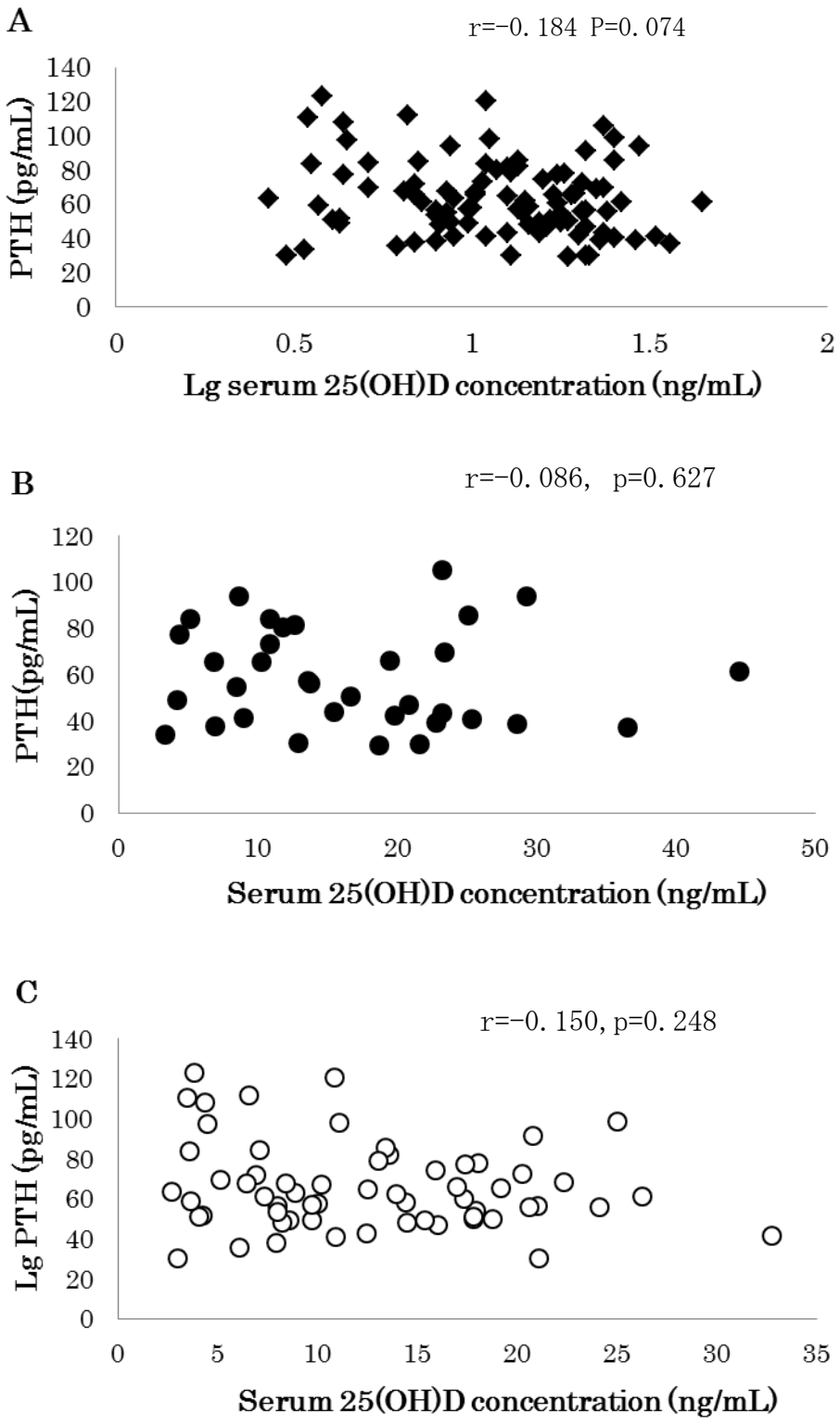

Figure 3-2 Relationships between serum 25(OH)D concentration and PTH.
In all adults(A), in male(B) and in female(C).

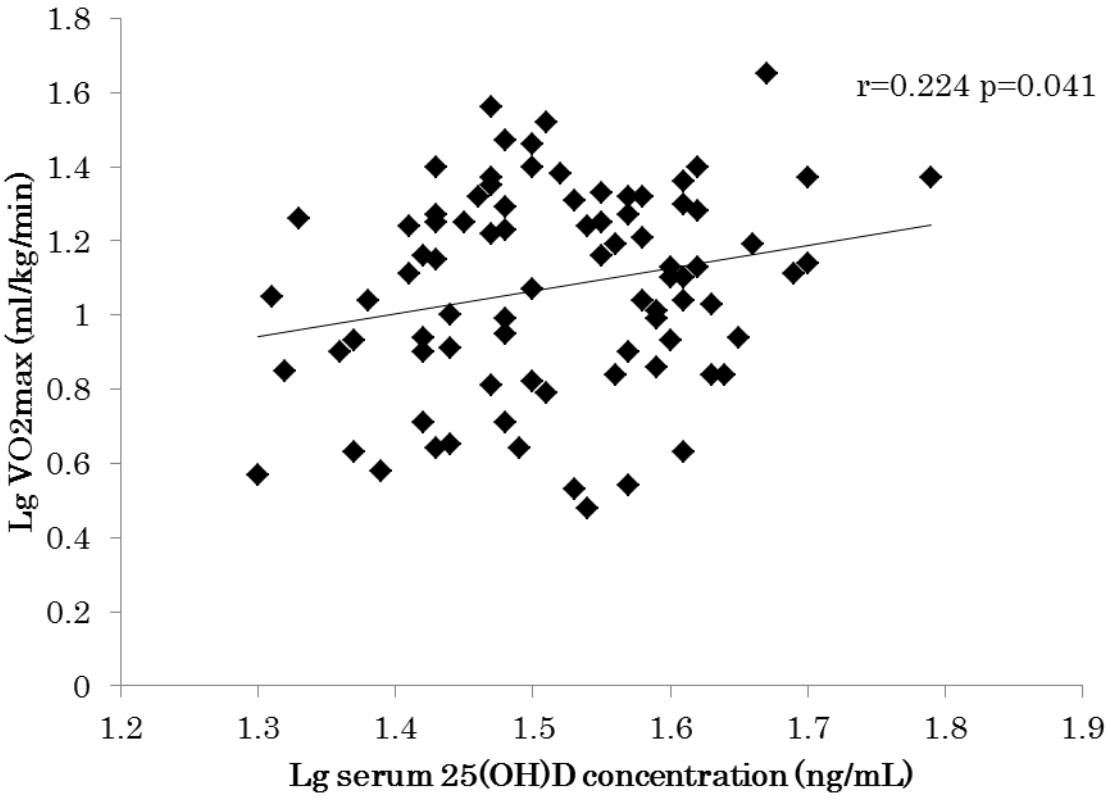

Figure 3-3 Relationship between serum 25(OH)D concentration and $\dot{V}O_2max$.

Table 3-2 Results from multiple linear regression analysis examining the association of serum 25(OH)D with VO_2max in adults.

	β	P	R^2
Model 1 Serum 25(OH)D (ng/mL) †	0.184	**0.039**	0.419
Model 2 Serum 25(OH)D (ng/mL) †	0.165	0.054	0.475
Model 3 Serum 25(OH)D (ng/mL) †	0.159	0.074	0.476

Model 1was adjusted for gender, age.

Model 2 was adjusted for gender, age, BMI.

Model 3 was adjusted for gender, age, BMI, MVPA.

MVPA, Moderate to vigorous physical activity.

† Log-transformed before performing the analysis.

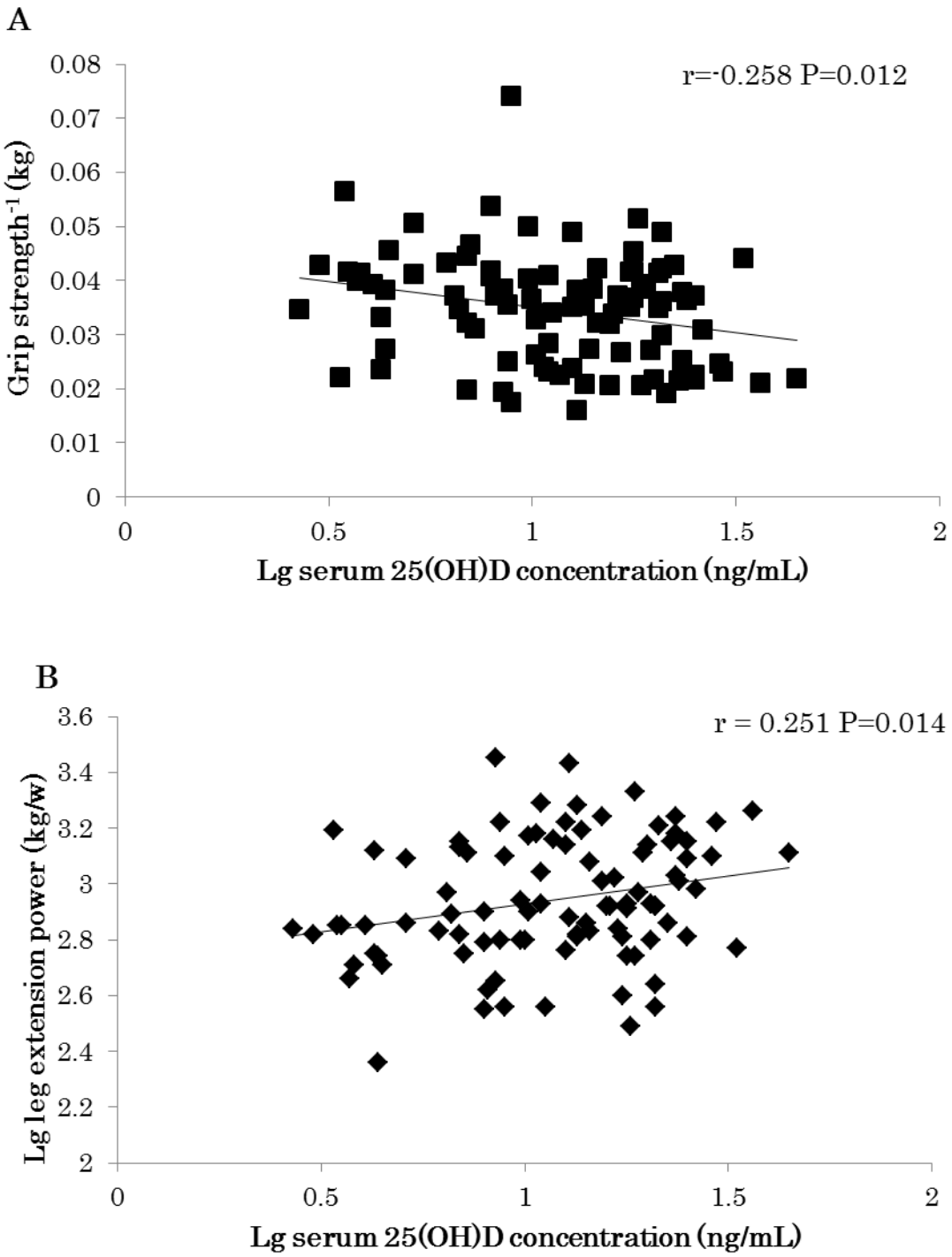

Figure 3-4 Relationships between serum 25(OH)D concentration and muscular strength.(A) hand grip strength and power (B) leg extension power.

Table 3-3 Results from multiple linear regression analysis examining the association of serum 25(OH) D with hand grip strength.

	β	P	R^2
Model 1 Serum 25(OH)D (ng/mL)	-0.137	**0.044**	0.662
Model 2 Serum 25(OH)D (ng/mL)	-0.148	**0.027**	0.680
Model 3 Serum 25(OH)D (ng/mL)	-0.168	**0.016**	0.685
Model 4 Serum 25(OH)D (ng/mL)	-0.149	**0.034**	0.693

Model 1 was adjusted for gender and age.

Model 2 was adjusted for gender, age and BMI.

Model 3 was adjusted for gender, age, BMI and MVPA.

Model 4 was adjusted for gender, age, BMI, MVPA and $\dot{V}O_2$max.

The dependent variable was grip strength transformed to X^{-1} before performing the analysis. Serum 25(OH)D concentration, MVPA and $\dot{V}O_2$max was transformed to log- before performing the analysis.

Table 3-4 Results from multiple linear regression analysis examining the association of serum 25(OH) D with leg extension power.

	β	P	R^2
Model 1 Serum 25(OH)D (ng/mL)	0.140	**0.041**	0.656
Model 2 Serum 25(OH)D (ng/mL)	0.154	**0.020**	0.686
Model 3 Serum 25(OH)D (ng/mL)	0.188	**0.006**	0.701
Model 4 Serum 25(OH)D (ng/mL)	0.140	**0.027**	0.748

Model 1 was adjusted for gender and age.

Model 2 was adjusted for gender, age and BMI.

Model 3 was adjusted for gender, age, BMI and MVPA.

Model 4 was adjusted for gender, age, BMI, MVPA and $\dot{V}O_2$max.

The dependent variable was leg extension power transformed to log- before performing the analysis.

Serum 25(OH)D concentration, MVPA and $\dot{V}O_2$max was transformed to log- before performing the analysis.

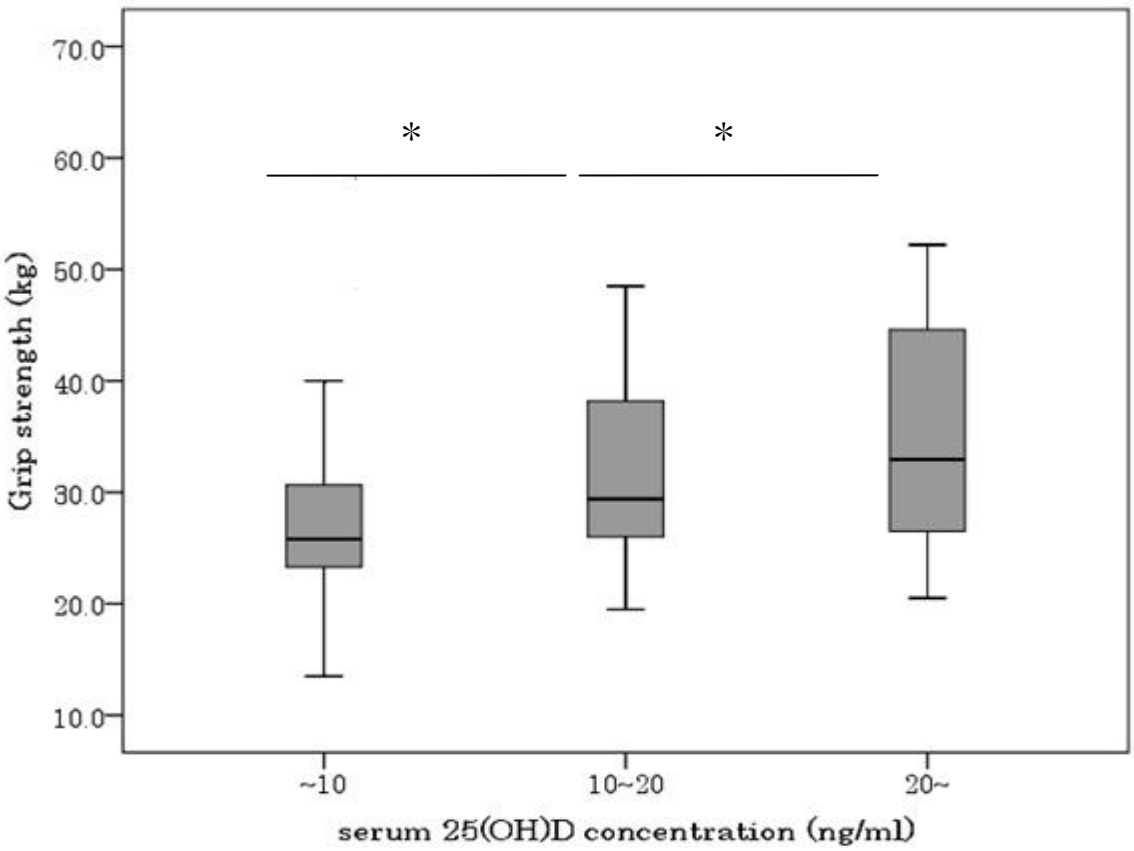

Figure 3-5 Comparison of mean hand grip strength of subjects in three serum 25(OH)D concentration categories (<10ng/mL, 10~20ng/mL, ≧20ng/mL). *p<0.05

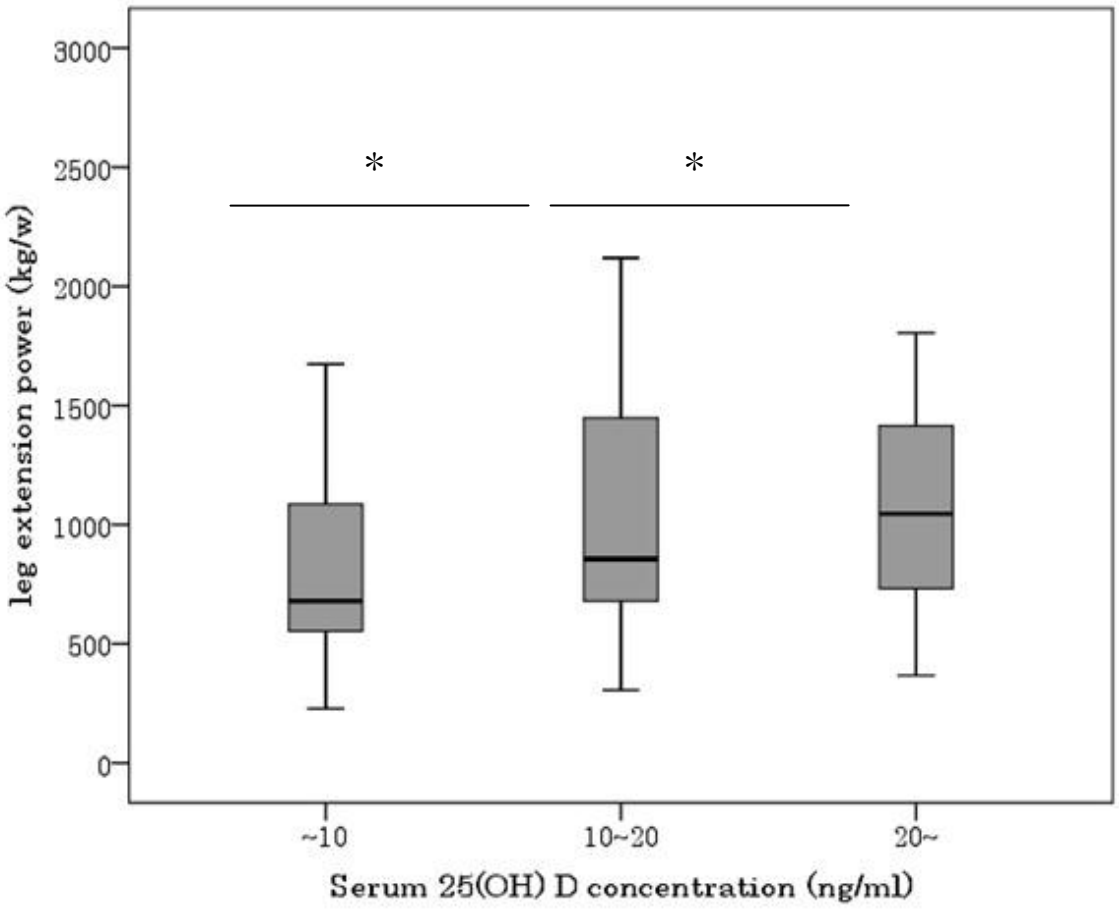

Figure 3-6 Comparison of mean leg extension power of subjects in three serum 25(OH)D concentration categories (<10ng/mL, 10~20ng/mL, ≧20ng/mL). $^{*}p<0.05$

Chapter 4: Discussion & Conclusion

4-1 Vitamin D Status in Japan

In this cross-sectional study of healthy Japanese adults, we found a high prevalence of vitamin D deficiency (84% in female and 65% in male), and serum 25(OH)D concentration in female was significantly lower than in male.

Studies in U.S. and Europe have shown that vitamin D levels vary by season and that the prevalence of vitamin D deficiency was higher during winter.[1,12-15,20-21)] In the present study, the prevalence of vitamin D deficiency was 78% in spring, in Saitama, Japan. Our data conform to the few Japanese studies that vitamin D deficiency is spreading in Japan. In a previous study in Japanese working population, it evidenced that the prevalence of vitamin D deficiency was 9.3%, respectively, in summer and 46.7% in late autumn, in Northeast Kyushu Japan.[32)] Another study of 197 adult men and women aged 20 to 68 years in the Tokai area (35.3°N) of Japan, found that the prevalence of vitamin D deficiency was 1%, respectively, in September and 26% in December.[59)] Another study reported that mean serum 25(OH)D concentration was 31.5ng/mL in summer and 23.9 ng/mL in winter among 122 adult women aged 45 to 81 years in the Hokuriku area(37.5°N) of Japan.[60)] Therefore, those prior studies, in conjunction with the present study , document that vitamin D deficiency was severe spread in Japan.

The data in the present study also showed that female had a lower mean serum 25(OH)D concentration than did male. This gender difference has been observed in many,[1)] but not all studies.[61-64)] Although the reason for the discrepancy is unclear, it probably reflects greater avoidance of sunlight among women. Sun avoidance includes the use of screens[65-66)] that effectively absorb ultraviolet β radiation, thus resulting in reduced vitamin D production in the skin.[67)] Gender differences in vitamin D status might also be due to differences in body composition profiles. Vitamin D is fat-soluble and is thus stored in fat tissue, the level of which is generally higher in women than in men.[68)] This results in lower serum 25(OH)D concentration in female.

4-2 Relationship of Vitamin D with Cardiorespiratory Fitness

Recently, vitamin D deficiency and low cardiorespiratory fitness are important independent risk factors for several chronic health conditions. To our knowledge, this study is the first large cross sectional study of the relation between serum 25(OH)D concentration and cardiorespiratory fitness among Japanese adults. In the present study, we documented that 25(OH)D is positive association with cardiorespiratory fitness($r=0.224$, $p=0.041$) and also existed after adjusting for gender and age ($\beta=0.184$,$p=0.039$). Moreover there was a borderline statistically significant relationship after adjusting further for BMI ($\beta=0.165$,$p=0.054$).But after further adjusting for MVPA ($\beta=0.159$ $p=0.074$), there were no statistically relationships.

There has been little published studies on the relation between cardiorespiratory fitness and serum

25(OH)D concentration. Mowry et al 2009 examined the association of cardiorespiratory fitness ($\dot{V}O_2$max) with 25(OH)D in 59 young healthy women 16 to 24 years old. They found that there was a positive association between $\dot{V}O_2$max and serum 25(OH)D($r=0.29, p<0.05$).[69] Ardestani A et al 2011 found that there was relation between 25(OH)D serum with $\dot{V}O_2$max ($r=0.29, p<0.0001$) in men and women over a broad age range (20 to 73 years) and serum 25(OH)D levels (10 to 82 ng/mL). They also found that the positive relation between 25(OH)D and $\dot{V}O_2$max persisted after adjustment for gender, age, BMI and MVPA.[44]

Cardiorespiratory fitness is determined primarily by maximal cardiac output and maximal arteriovenous O_2 difference. Consequently, while precise mechanisms by which serum 25(OH)D levels may affect cardiorespiratory fitness levels have not been identified, possible mechanisms that should be investigated include alterations in the following: maximal HR and maximal stroke volume, as well as skeletal muscle characteristics, such as fiber type, myoglobin content, mitochondrial size, number, and function, calcium-troponin binding kinetics, and motor unit recruitment. Thus, vitamin D role's in cardiorespiratory fitness can be explained that muscle and cardiac mitochondrial function and oxygen consumption are factors. Cardiac myocytes possess the vitamin D receptor[70] and have vitamin D-dependent oxidative phosphorylation, such that ATP production is reduced in states of hypovitaminosis D to levels that are 25% lower than controls.[71] Our data was showed that there was a borderline statistically significant relation between serum 25(OH)D with $\dot{V}O_2$max. Therefore, we can consider that the relationship may be existed in Japanese adults.

Despaired of our expectation, results of this study did not show the relationship between serum 25(OH)D with $\dot{V}O_2$max after adjusting for MVPA. As one explanation, it is maybe that serum 25(OH)D was not independently related to $\dot{V}O_2$max, and affected by BMI and MVPA. It has been considered that cardiorespiratory fitness is simply a surrogate for a subject's daily physical activity, which could be related to light exposure and therefore to serum 25(OH)D. In prior studies, Ardestani A et al did find an interaction of 25(OH)D level with the amount of physical activity such that subjects with lowest level of MVPA demonstrated the strongest relation between 25(OH)D and $\dot{V}O_2$max. Each SD increase in serum 25(OH)D increased $\dot{V}O_2$max by 8%(with change calculated compared to the group average) in those with the lowest level of MVPA. And SD increase in serum 25(OH)D also increased $\dot{V}O_2$max by 5% in those with a moderate level of MVPA but only by 0.2% in those with a high level of MVPA. [44]

The mechanism for the interactive effect of 25(OH)D and MVPA on cardiorespiratory fitness is not clear. $\dot{V}O_2$max is limited by cardiac output, arterial oxygen content, shunting of blood to active muscle, and extraction of oxygen by these muscles. Low serum 25(OH)D concentration can cause myocardial hypertrophy, increased blood pressure, and endothelial dysfunction by 25(OH)D receptors.[1,31,72-74] Consequently, low serum 25(OH)D concentration may decrease cardiac output and increase peripheral vessel resistance, decreasing $\dot{V}O_2$max. Physical activity is also known to increase

$\dot{V}O_2$max through increased cardiac output.[75-77] Results from a large healthy adult cohort study have suggested that the greatest benefits of physical activity on cardiac remodeling occur at the lowest levels of reported physical activity.[78] Therefore, serum 25(OH)D could potentially have a greater benefit on cardiac remodeling and $\dot{V}O_2$max in subjects with low levels of physical activity than in those who already engage in high levels of activity. There is also evidence that physical inactivity[79,80] and vitamin D deficiency[53-54,81] can cause muscle atrophy and shift muscle fiber type from IIa to IIb. Therefore, subjects with the lowest level of physical activity may receive a greater aerobic benefit from increasing serum 25(OH)D concentration by changes in muscle mass and fiber type than those who already engage in high levels of physical activity.

For another explanation, it was considered that sample size was small in the present study. Our data showed that there was a borderline statistically significant relationship of serum 25(OH)D with $\dot{V}O_2$max after adjusting for gender, age and BMI($p=0.054$), so we had to deliberately consider that maybe the effect of serum 25(OH)D on $\dot{V}O_2$max would be obvious with increasing sample size.

4-3 Relationship of Vitamin D with Muscular Strength

To our knowledge the first large cross sectional study investigating the relationship of vitamin D status with hand grip strength and leg extension power among Japanese adults. We have evidenced that higher vitamin D levels are associated with both hand grip strength ($p=0.026$) and leg extension power ($p=0.017$), and the relationships were also considerably existed after adjusting for gender, age, BMI, MVPA and $\dot{V}O_2$max.

There have been some studies examining the relationship between vitamin D status and muscular strength of the hand. Taes YE et al 2006 found that serum 25(OH)D concentrations correlated directly with biceps force($p=0.024$) in 211 elderly male between the ages of 71 and 86.[82] Oh JH et al 2009 found that serum 25(OH)D levels were also directly related to abduction($p=0.001$) and external rotation ($p<0.001$) torque of shoulder muscles in men and women with rotator cuff disorders.[83] Similarly, another studies found that serum 25(OH)D was directly related to hand grip strength in 435 men ($p=0.004$) and 541 women ($p=0.01$) $\geq$ 65 years of age[84] as well as among 70 women ($p<0.05$) over the age of 65 whose 25(OH)D concentration ranged from 8 to 20 ng/mL.[85] The rare study examining individuals across the adult age range has failed to observe a consistent relationship between serum 25(OH)D concentration and upper body strength, measured as hand grip strength.[86] Conforming to prior studies, we observed there was significant positive correlation between serum 25(OH)D concentration and hand grip strength($p=0.012$). In addition, the relationship remained after adjusted by gender, age ($\beta=-0.137$,$p=0.044$), BMI($\beta=-0.148$,$p=0.027$), moderate to vigorous physical activity($\beta=-0.168$, $p=0.016$) and $\dot{V}O_2$max($\beta=-0.149$,$p=0.034$). This was evidenced that serum 25(OH)D concentration contributes independently to hand grip strength.

There are also few studies examining the relationship between vitamin D status and leg extension power. Gerdhem P et al 2005 found that serum 25(OH)D concentration less than 30 ng/mL were

associated with poor thigh isometric extension (p=0.020) and flexion (p=0.032) performance in women 75 years of age.[87] Our data has shown a direct relation between serum 25(OH)D concentration with leg extension power among Japanese adults(r=0.251,p=0.014). Moreover, there was significant positive correlation between serum 25(OH)D concentration and leg extension power (p=0.014). And the relationship remained after adjusted by gender, age (β=0.140, p=0.041), BMI(β=-0.154,p=0.020), moderate to vigorous physical activity(β=0.188, p=0.006) and $\dot{V}O_2$max(β=0.140,p=0.027) (Table 3-3). Thus, we have also documented that serum 25(OH)D concentration is independently related to leg extension power.

The mechanism of the relation between serum 25(OH)D and muscle strength is unclear. Vitamin D deficiency affects type II skeletal muscle fibers and can produce a myopathy. Type II muscle fiber atrophy of the intercostals muscles has been reported in two cases of patients with osteomalacic myopathy and vitamin D levels less than 30 ng/mL.[88] Middle gluteal muscle samples from elderly women with vitamin D deficiency (<15.6 ng/mL) also show type II muscle fiber atrophy compared to individuals with higher vitamin D levels (>15.6 ng/mL). Furthermore, when serum 25(OH)D concentration was less than 15.6 ng/mL, the mean type II fiber diameter correlated with vitamin D status (r= 0.714, p=0.0011).[89] Similarly, when 1000 IU of vitamin D were provided daily for 2 years to 96 elderly women with vitamin D deficiency (<10 ng/mL) in a randomized, placebo controlled trial, the type II muscle fibers of the vastus lateralis in the vitamin D group increased an average of 96.5% in diameter whereas the untreated group experienced a 22.5% decrease in diameter (p<0.0001), and the diameter of type II muscle fibers correlated with serum 25(OH)D concentration (r=0.558, p<0.0001).[90] There was also a 59% reduction in falls with vitamin D supplementation in that study. [90] Such results suggest that the effect of vitamin D may vary with the fiber type of the muscle, but also suggest that vitamin D supplementation may have important clinical effects.

It is also suggested $1,25(OH)_2D$ exerts its principal actions by binding to the VDR which has been identified in muscle tissues. $1,25(OH)_2D$ via intranuclear VDR activates gene transcription which results in the synthesis of specific proteins believed to influence muscle calcium handling, phosphate transport across the cell membrane, and muscle cell differentiation and proliferation.[91] Recently, another pathway via nongenomic mechanisms are probably occurring.[91]

Thus, the first limitation of this study was the relatively small size of the participant sampled. Second, only two adults in this study are obese (BMI >30kg/m^2). Therefore, our results do not necessarily apply to an obese population. Third, this study is the use of a cross sectional study design, longitudinal studies are required for the causal relationship. In future, we expect that future studies investigating the relationship between serum 25(OH)D and cardiorespiratory fitness are encouraged to include a larger sample size and longitudinal studies are required for causal relationship.

4-4 Conclusion

In conclusion, the high prevalence of vitamin D deficiency was found in our studies. We found that serum 25(OH)D concentration was related to cardiorespiratory fitness, but the relationship was affected by BMI and MVPA. Moreover, the present study also demonstrated that serum 25(OH)D are independently and positively associated with muscle strength, including hand grip strength and leg extension power in Japanese adults.

Acknowledgements

I appreciate all members of Higuchi laboratory for their encouragement and support in my master time at Higuchi laboratory in Waseda University. Especially, I would like to thank Dr. Mitsuru Higuchi, who has provided great support and encouragement for me. And, I would like to thank Dr. Hiroshi Kawano for always providing me a support and a guide.

I would like to say “Thank you” to my parents for their great admiration on my own freedom.

Lastly, I would like to express my deepest thanks to Dr. Zhen-Bo Cao for providing continued encouragement and teaching throughout my studies.

The study was supported by Grants-in-Aid for Scientific Research (C) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (no.23500864 to Dr. Zhen-Bo Cao).

Reference

1) Holick MF. Vitamin D deficiency. *N Engl J Med* 2007;19:266-81.
2) Pilz et al. Low serum levels of 25-hydroxyvitamin D predict Fata cancer in patients referred to coronary angiography. *Cancer Epidemiol Biomarkers Prev* 2008; 17(5):1228-33.
3) Wehr et al. Association of hypovitaminosis D with metabolic disturbances in polycystic ovary syndrome. *Eur J Endocrinol* 2009; 161(4): 575-82.
4) Lips P. Which circulating level of 25-hydroxyvitamin D is appropriate? *J Steroid Biochem Mol Biol* 2004; 89-90(1-5): 611-4.
5) Heaney RP. Vitamin D and calcium interactions: functional outcomes. *Am J Clin Nutr* 2008; 88: 541-4.
6) Vin Tangpricha. Vitamin D in food and supplements. *Am J Clin Nutr* 2012; 95(6): 1299-300.
7) Bouillon R, Van Cromphaut S, Carmeliet G. Intestinal calcium absorption: Molecular vitamin D mediated mechanisms. *J Cell Biochem* 2003; 88(2):332-9.
8) Holick MF, Garabedian M. Vitamin D:photobiology, metabolism, mechanism of action, and clinical applications. In: Favus MJ, ed. Primer on the metabolic bone diseases and disorders of mineral metabolism. 6th ed. Washington, DC: American Society for Bone and Mineral Research 2006; 129-37.
9) Bouillon R. Vitamin D: from photosynthesis, metabolism, and action to clinical applications. In: DeGroot LJ, Jameson JL, eds. Endocrinology. Philadelphia: W.B. Saunders 2001; 1009-28.
10) DeLuca HF. Overview of general physiologic features and functions of vitamin D. *Am J Clin Nutr* 2004; 80: Suppl; 1689S-1696S.
11) Holick MF. Sunlight and vitamin D for bone health and prevention of autoimmune disease, cancers, and cardiovascular disease. *Am J Clin Nutr* 2004; 80(6 Suppl): 1678S-88S.
12) Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B. Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr* 2006; 34:18-28.
13) Thomas KK, Lloyd-Jones DM, Thadhani RI, et al. Hypovitaminosis D in medical inpatients. *N Engl J Med* 1998; 338: 777-83.
14) Chapuy MC, Preziosi P, Maamer M, et al. Prevalence of vitamin D insufficiency in an adult normal population. *Osteoporos Int* 1997; 7: 439-43.
15) Holick MF, Siris ES, Binkley N, et al. Prevalence of vitamin d inadequacy among postmenopausal North American women receiving osteoporosis therapy. *J Clin Endocrinol Metab* 2005; 90: 3215-24.
16) Nancy K. Latham, Crag S. Anderson, Ian R. Reid. Effects of vitamin D supplementation on strength, physical, performance, and falls in older persons: a systematic review. *J Am Geriatr Soc* 2003; 51:1219-1226.

17) Elena D. Toffanello, Egle P, Giuseppe S, Sabina Z, Estella M, Stefania M, Alessandra C, Leonardo S, Maria-Chiara C, Giovannella B, Gaetano C, Enzo M. Vitamin D and physical performance in elderly subjects: the Pro.V.A study. *Plos One* 2012;7(4):e34850.

18) Puchacz E, Stumpf WE, Stachowiak EK, Stachowiak MK. Vitamin D increases expression of the tyrosine hydroxylase gene in adrenal medullary cells. *Brain Res Mol Brain Res* 1996; 36(1): 193-6.

19) Lisa Ceglia. Vitamin D and skeletal muscle tissue and function. *Mol Aspects Med* 2008; 29:407-414.

20) Holick MF. High prevalence of vitamin D inadequacy and implication for health. *Mayo clin Proc* 2006; 81:353-73.

21) Malabanan A, Veronikis IE, Holick MF. Redefining vitamin D insufficieny. *Lancet* 1998; 351:805-6.

22) Heaney RP, Dowell MS, Hale CA, Bendich A. Calcium absorption varies within the reference range or serum 25-hydroxyvitamin D. *J Am Coll Nutr* 2003; 22: 142-6.

23) Dawson- Hughes B, Heaney RP, Holick MF, Lips P, Meunier PJ, Vieth F. Estimates of optimal vitamin D status. *Osteoporos Int* 2005; 16: 713-6.

24) Cranney A, Horsley T, O'Donnell S, et al. Effectiveness and safety of vitamin D in relation to bone health. *Evid Rep Technol Assess* 2007; 158:1-235.

25) Bischoff-Ferrari HA, Willett WC, Orav EJ,et al. A pooled analysis of vitamin dose requirements for fracture prevention. N. *Engl. J. Med* 2012 367(1): 40-9.

26) Buttigliero C, Monagheddu C, Petroni P, Saini A, Dogliotti L, Ciccone G, Berruti A. Prognostic role of vitamin D status and efficacy of vitamin D supplementation in cancer patients; a systematic review. *Oncologist* 2011; 16(9): 1215-27.

27) Pittas AG, Chung M, Trikalinos T, Mitri J, Brendel M, Patel K, Lichtenstein AH, Lau J, Balk EM. Vitamin D and Cardio metabolic Outcomes: A Systematic Review. *Ann Intern Med* 2010; 152(5):307-14.

28) Wang L, Manson JE, Song Y, Sesso HD. Systematic review:Vitamin D and calcium supplementation in prevention of cardiovascular events. *Ann Intern Med* 2010;152(5):315-23.

29) Chiu KC, Chu A, Go VLW, Saad MF. Hypovitaminosis D is associated with insulin resistance and β cell dysfunction. *Am J Clin Nutr* 2004; 79: 820-5.

30) Zittermann A. Vitamin D and disease prevention with special reference to cardiovascular disease. *Prog Biophys Mol Biol* 2006; 92(1): 39-48.

31) Zittermann A, Schleithoff SS, Tenderich G, Berthold HK, Korfre R, Stechle P. Low vitamin D status: a contributing factor in the pathogenesis of congestive heart failure? *J Am Coll Cardiol* 2003; 41: 105-12.

32) Akiko N, Leng HF, Kazutoshi N, Ai H, Kalpana PT, Yumi M, Tetsuya M. Serum

25-hydroxyvitamin D concentrations and season-specific correlates in Japanese adults. *J Epidemiol* 2011; 21(5): 346-53.

33) Kumihiro M, Tokuo M, Shigeru S, Kenji F. Prevalence and risk factors of vitamin D deficiency rickets in Hokkaido, Japan. *Pediatr Int* 2009; 51: 559-62.

34) Caspersen CJ, Powell KE, Christenson GM. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. *Public Health Rep* 1985;100(2):126-31.

35) Donatello, Rebeka J. Health, The Basics. *San Francisco: Pearson Education, Inc*. 2005.

36) Blair SN, Kohl HW III, Pafenbarger RS Jr, Clark DG, Cooper KH, Gibbons LW. Physical fitness and all-cause mortality: a prospective study of healthy men and women. *JAMA* 1989; 262(17): 2395-401.

37) Dobnig H, Pilz S, Scharnagl H, et al. Independent association of low serum 25-hydroxyvitamin d and 1,25-dihydroxyvitamin D levels with all-case and cardiovascular mortality. *Arch Intern Med* 2008; 168(11): 1174-80.

38) Melamed ML, Michos ED, Post W, Asotr B. 25-hydroxyvitamin D levels and the risk of mortality in the general population. *Arch Intern Med* 2008; 168(15): 1629-37.

39) Zittermann A, Gummert JF, Borgermann J. Vitamin D deficiency and mortality. *Curr Opin Clin Nutr Metab Care* 2009; 12(6): 634-9.

40) Nibbelink KA, Tishkoff DX, Hershey SD, Rahman A, Simpson RU. 1,25(OH)2-vitamin D_3 actions on cell proliferation, size, gene expression, and receptor localization, in the HL-1 cardiac myocyte. *J Steroid Biochem Mol Biol* 2007; 103(3-5): 533-7.

41) Stephen WF, Joseph PC, Benjamin LW. Cardiorespiratory Fitness, adiposity, and serum 25-dihydroxyvitamin D levels in men. *Med Sci Sports Exerc* 2011; 43(2):266-71.

42) Farell SW, Willis BL. Cardiorespiratory fitness, adiposity, and serum 25-dihydroxyvitamin D levels in women: the Cooper Center Longitudinal Study. *J Womens Health (Larchmt)* 2012; 21(2): 80-6.

43) Debra AM, Megan MC, Kate AH. Association among cardiorespiratory fitness, body fat, and bone marker measurements in healthy young females. *J Am Osteopath Assoc* 2009; 109(10):534-9.

44) Ardestani A, Parker B, Mathur S, Clarkson P, Pescatello LS, Hoffman HJ, Polk DM, Thompson PD. Relation of vitamin D level to maximal oxygen uptake in adults. *Am J Cardiol* 2011; 107(8): 1246-9.

45) Jurca R, Lamonte MJ, Barlow Ce, Kampert JB, Church TS, Blair SN. Association of muscular strength with incidence of metabolic syndrome in men. *Med Sci Sports Exerc* 2005; 37(11):1849-55.

46) Wicherts IS, van Schoor NM, Boeke AJ et al. Vitamin D status predicts physical performance and its decline in older persons. *J Clin Endocrinol Metab* 2007; 92: 2058-2065.

47) Bischoff-Ferrari HA, Dietrich T, Orav EJ, Hu FB, Zhang Y, Karison EW, Dawson-Hughes B.

Higher 25-hydroxyvitamin D concentrations are associated with better lower-extremity function in both active and inactive persons aged>or = 60y. *Am J Clin Nutr* 2004; 80: 752-8.

48) Kuchuk NO, Pluijm SM, et, al. Relationships of serum 25-hydroxyvitamin D to bone mineral density and serum parathyroid hormone and markers of bone turnover in older persons. *J Clin Endocrinol Metab* 2009; 94: 1244-50.

49) Houston DK, Tooze JA, Davis CC, Chaves PH, Hirsch CH, Robbins JA, Arnold AM, Newman AB, Kritchevsky SB. Serum 25-hydroxyvitamin D and physical function in older adults: the Cardiovascular Health Study All Stars. *J Am Geriatr Soc* 2011; 59: 1793-801.

50) Ward KA, Das G, Berry JL, Roberts SA, Rawer R, Adams JE, Mughal Z. Vitamin D status and muscle function in postmenarchal adolescent girls. *J Clin Endocrinol Metab* 2009; 94: 559-63.

51) Barker T, Martin TB, Hill HR, Kjeldsberg CR, Henriksen VT, Dixon BM, Schneider ED, Dern A, Weaver LK. Different doses of supplemental vitamin D maintain interleukin-5 without altering skeletal muscle strength: a randomized, double-blind, placebo-controlled study in vitamin D sufficient adults. *Nutr Metab(Lond)* 2012; 9(1): 16.

52) Grimaldi AS, Parker BA, Capizzi JA, Clarkson PM, Pescatello LS, White MC, Thompson PD. 25(OH) vitamin D is associated with greater muscle strength in healthy men and women. *Med Sci Sports Exerc* 2012; 45(1): 157-62.

53) Bischoff-Ferrari HA, Borchers M, Gudat F, Durmuller U, Stahelin HB, Kick W. Vitamin D receptor expression in human muscle tissue decreases with age. *J Bone Miner Res* 2004; 19: 265-9.

54) Sorensen OH, Lund B, Saltin B, Lund B, Andersen RB, Hjorth L, et al. Myopathy in bone loss of ageing: improvement by treatment with 1 alpha- hydroxycholecalciferol and calcium. *Clin Sci (Lond)* 1976; 56: 157-61.

55) Okunno J, Tomura S, Yabushita N, Kim MJ, Okura T, Tanaka K, Yanagi H. Effects of serum 25-hydroxyvitamin D(3) levels on physical fitness in community-dwelling frail women. *Arch Gerontol Geriatr* 2010; 50(2): 121-6.

56) Cao ZB, Miyatake N, Higuchi M, Ishikawa-Takata K, Miyachi M, Tabata I. Prediction of VO_2max with daily step counts for Japanese adult women. *Eur J Appl Physiol* 2009; 105(2): 289-96.

57) Cao ZB, Miyatake M, Higuchi M, Miyachi M, Tabata I. Predicting VO_2max with an objectively measured physical activity in Japanese men. *Eur J Appl Physiol* 2010; 109(3): 465-72.

58) Gutin B, Yin Z, Humphries MC, Barbeau P. Relations of moderate and vigorous physical activity to fitness and fatness in adolescents. *Am J Clin Nutr* 2005; 81(4): 746-750.

59) Ono Y, Suzuki A, Kotake M, Zhang X, Nishiwaki-Yasuda K, Ishiwata Y, et al. Seasonal changes of serum 250hydroxyvitamin D and intact parathyroid hormone levels in a normal Japanese population. *J Bone Miner Metab* 2005; 23: 147-51.

60) Nakamura K, Nashimoto M, Yamamoto M. Summer/winter differences in the serum 25-hydroxyvitamin D3 and parathyroid hormone levels of Japanese women. *Int J Biometeorol* 2000; 44: 186-9.

61) Hintzpeter B, Mensink GB, Thierfelder W, Muller MJ, Scheidtnave C. Vitamin D status and health coreelates among German adults. *Eur J Clin Nutr* 2008; 62: 1079-89.

62) Brock K, Huang WY, Fraser Dr, Ke L, Tseng M, Stolzenberg SR, et al. Low vitamin D status is associated with physical inactivity, obesity and low vitamin D intake in a large US sample of healthy middle-aged men and women. *J Steroid Biochem Mol Biol* 2010; 121: 462-6.

63) Burnand B, Sloutskis D, Gianoli F, Cornuz J, Rickenbach M, Paccaud F, et al. Serum 25-hydroxyvitamin D: distribution and determinants in the Swiss population. *Am J Clin Nutr* 1992; 56: 537-42.

64) Van der Meer IM, Boeke AJ, Lips P, Grootjans-Geerts I, Wuister JD, Deville WL, et al. Fatty fish and supplements are the greatest modifiable contributors to the serum 25-hydroxyvitamin D concentration in a multiethnic population. *Clin Endocrinol (Oxf)* 2008; 68: 466-72.

65) Cheng S, Lian S, Hao Y, Kang N, Li S, Nie Y, et al. Sun exposure knowledge and protection behavior in a Northe Chinese population: a questionnaire-based study. *Photodermatol Photoimmunol Photomed* 2010; 26: 177-81.

66) Jerkegren E, Sandrieser L, Brandberg Y, Rosdahl I. Sun-related behavior and melanoma awareness among Swedish university students. *Eur J Cancer Prev* 1999; 8: 27-34.

67) Holick MF. Photobiology of vitamin D. In: Feldman D, Pike JW, Glorieux FH, editors. Vitamin D second edition. *London: Elsevier Academic Press;* 2005.p. 37-45.

68) Rahmaniyan M, Bell NH, editors. Vitamin D second edition. *London: Elsevier Academic Press;* 2005.p. 789-801.

69) Mowry DA, Costello MM, Heelan KA. Association among cardiorespiratory fitness, body fat, and bone marker measurements in healthy young females. *J Am Osteopath Assoc* 2009; 109: 534-9.

70) Koleganova N, Piecha G, Ritz E, Gross ML. Calcitriol ameliorates capillary deficit and fibrosis of the heart in subtotally nephrectomized rats. *Nephrol Dial Transplant* 2009; 24: 778-87.

71) Mukherjee A, Zerwekh JE, Nicar MJ, McCoy K, Buja LM. Effect of chronic vitamin D deficiency on chick heart mitochondrial oxidative phosphorylation. *J Mol Cell Cardiol* 1981; 13: 171-83.

72) Gouni-Berthold I, Krone W, Berthold HK. Vitamin D and cardiovascular disease. *Curr Vasc Pharmacol* 2009; 7: 414-22.

73) Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the rennin-angiotensin system. *J Clin Invest* 2002; 110: 229-38.

74) Mitsuhashi T, Morris RC Jr, Ives HE. 1,25-Dihydroxyvitamin D3 modulates growth of

vascular smooth muscle cells. *J Clin Invest* 1991; 87: 1889-95.

75) Gormley SE, Swain DP, High R, Spina RJ, Dowling EA, Kotipalli US, Gandrakota R. Effect of intensity of aerobic training on VO_2max. *Med Sci Sports Exerc* 2008; 40: 1336-43.

76) Bassett DR Jr, Howley ET. Limiting factors for maximum oxygen uptake and determinants of endurance performance. *Med Sci Sports Exerc* 2000; 32: 70-84.

77) Mitchell JH, Blomqvist G. Maximal oxygen uptake. *N Engl J Med* 1971; 284: 1018-22.

78) Turbey EB, Jorgensen NW, Johnson WC, Bertoni AG, Polak JF, Roux AV, Tracy RP, Lima JA, Bluemke DA. Physical activity and physiological cardiac remodeling in a community setting: the Multi-Ethnic Study of Atherosclerosis(MESA). *Heart* 2010; 96: 42-8.

79) Haggmark T, Eriksson E. Hypotrophy of the soleus muscle in man after Achilles tendon rupture. Discussion of findings obtained by computed tomography and morphologic studies. *Am J Sports Med* 1979; 7: 434-48.

80) Andersen P, Henriksson J. Training induced changes in the subgroups of human type II skeletal muscle fiber. Acta Physiol Scand 1977; 99: 123-5.

81) Boland R. Role of vitamin D in skeletal muscle function. *Endocr Rev* 1986; 7: 434-48.

82) Taes YE, Goemaere S, Huang G, Van Pottelbergh I, De Bacquer D, Verhasselt B, Van den Broeke C, Delanghe JR, Kaufman JM. Vitamin D binding protein, bone status and body composition in community- dwelling elderly men. *Bone* 2006; 38(5): 701-7.

83) Oh JH, Kim SH, Kim JH, Shin YH, Yoon JP, Oh CH. The level of vitamin D in the serum correlates with fatty degeneration of the muscles of the rotator cuff. *J Bone Joint Surg Br* 2009; 91(12): 1587-93.

84) Houston DK, Cesari M, Ferrucci L, Cherubini A, Maggio D, Bartali B, Johnson MA, Schwartz GG, Krichevsky SB. Association between vitamin D status and physical performance: the InCHIANTI study. *J Gerontol A Biol Sci Med Sci* 2007; 62(4): 440-6.

85) Janssen HC, Samson MM, Verhaar HJ. Muscle strength and mobility in vitamin D insufficient female geriatric patients: a randomized controlled trial on vitamin D and calcium supplementation. *Aging Clin Exp Res* 2010; 22(1): 78-84.

86) Ceglia L, Chiu GR, Harris SS, Araujo AB. Serum 25-hydroxyvitamin D concentration and physical function in adult men. *Clin Endocrinol (Oxf)* 2011; 74(3): 370-6.

87) Gerdhem P, Ringsberg KA, Obrant KJ, Akesson K. Association between 25-hydroxyvitamin D levels, physical activity, muscle strength and fractures in the prospective population-based OPRA study of elderly women. *Osteoporos Int* 2005; 16(11): 1425-31.

88) Yoshikawa S, Nakamura T, Tanabe H, Imamura T. Osteomalacic myopathy. *Endocrinol Jpn* 1979; 26(Suppl): 65-72.

89) Sato Y, Inose M, Higuchi I, Higuchi F, Kondo I. Changes in the supporting muscles of the fractured hip in elderly women. *Bone* 2002; 30(1): 325-30.

90) Sato Y, Iwamoto J, Kanoko T, Satoh K. Low-dose vitamin D prevents muscular atrophy and reduces falls and hip fractures in women after stroke: a randomized controlled trial. *Cerebrovasc Dis* 2005; 20(3): 187-92.

91) Ceglia L, Harris SS. Vitamin D and its role in skeletal muscle. *Calcif Tissue Int* 2012.