2012 年度　修士論文

血中ビタミン D 濃度と炎症マーカーとの関係

Association between serum 25-hydroxy vitamin D and inflammatory markers

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5011A043-2

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Abbreviations

AI adequate intake
AODM adult-onset diabetes mellitus
AT adiposity tissue
BW body weight
BMI body mass index
CHD coronary heart disease
CV coefficient of variation
CVD cardiovascular disease
CRP C-reactive protein
CYP24 cytochrome P450 24-hydroxylase
CYP27 cytochrome P450 27-hydroxylase
DRIs dietary reference intakes
DXA dual energy x-ray absorptiometry
ELISA enzyme linked immunosorbent assay
FEV1 forced expiratory volume in 1 second
IOM institute of medicine
IU international unit
IFN-γ interferon-gamma
IL-6 interleukin-6
IL-17 interleukin-17
KO knockout mice
MVPA moderate to vigorous physical activity
MS multiple sclerosis
NO nitric oxide
NHANES III Third National Health and –
-Nutrition Examination Survey
OZ ounces
PTH parathyroid hormone
PA physical activity
PBMCs peripheral blood mononuclear cells
RA rheumatoid arthritis
RDA recommended dietary allowance
SAA serum amyloid A
SVD serum vitamin D
TB tuberculosis
Th T helper
T2D type 2 diabetes
TNF-α tumor necrosis factor-alpha
UV ultraviolet
URI upper respiratory infection
VDR vitamin D receptor
VPA vigorous physical activity
25(OH)D 25-hydroxy vitamin D
$1,25(OH)_2D$ 1,25-dihydroxyvitamin D

Content of Supplemental Figures and Tables

Introduction

During the past decade, important advances in the study of vitamin D have been made. In addition to its important role in skeletal development and maintenance, evidence is mounting that vitamin D produces beneficial effects on extraskeletal tissue. At the same time, numerous reports have shown that relatively high proportions of people have insufficient levels of vitamin D.

The term 'vitamin D' specially refers to the parental vitamin D produced endogenously by the action of sunlight on 7-dehydrocholesterol in skin (also known as vitamin D_3, or cholecalciferol), or obtained from dietary foodstuffs as either vitamin D_3 or vegetable vitamin D_2 (also known as ergocalciferol). 90% to 95% of our vitamin D requirement comes from exposure to sunlight (wavelengths of 290-315nm) [1]. Most experts agree that in the absence of sun exposure, at least 1000 IU and preferably 2000 IU of vitamin D/day is required to satisfy the adult's vitamin D need [2]. However dietary sources of vitamin D are limited. They include oily fish such as salmon, mackerel, and sardines; some foods are fortified in the United States, including milk and some cereals, orange juice, some yogurts and margarine, but such fortified food is less in Japan [3]. (Supplemental table 1)

Ultraviolet (UV)-B irradiation of skin triggers photolysis of 7-dehydroxycholesterol (provitamin D) to previtamin D in the plasma membrane of human skin keratinocytes. Once formed in the skin, cell plasma membrane previtamin D is rapidly converted to vitamin D by the skin's temperature. Then vitamin D is firstly converted to 25-hydroxy vitamin D (25[OH]D) in the liver which is the main circulating form of vitamin D used to define 'vitamin D status'. At physiological concentrations, 25(OH)D appears to be inactive as a signaling molecule. Consequently, the target cell function of vitamin D is

determined by conversion of 25(OH)D to active 1, 25-dihydroxyvitaminD (1,25[OH]$_2$D), which is mainly catalyzed by the vitamin D-activating enzyme 25-hydroxy vitamin D-1α-hydroxylase (CYP27B1) in the kidney. The 1,25(OH)$_2$D produced in this manner then functions as a steroid hormone by binding to the nuclear vitamin D receptor (VDR) and acting as a regulator of gene transcription. [4, 5] (Supplemental figure 1) Vitamin D derived from sunlight or diet typically occurs only intermittently. Irregular intake of vitamin D, irrespective of the source, can lead to chronic vitamin D deficiency.

Physical factors that attenuate UV-B exposure, including clothing, sunscreens, and glass shielding, markedly reduce or completely eliminate the production of vitamin D_3 in the skin. At latitudes above 35° N and below 35° S, sunlight is insufficient to induce cutaneous vitamin D_3 synthesis during the winter months. [6] Biological factors that inhibit cutaneous vitamin D synthesis and bioavailability include skin pigmentation [6, 7], medication use [8], body fat content [9], fat malabsorption [10], and age [11]. An increase in skin pigmentation or the topical application of a sunscreen will absorb solar UVB photons, thereby significantly reducing the production of vitamin D3 in the skin by as much as 99 % [7, 14]. Certain drugs (such as anticonvulsants, corticosteroids and rifampin) may adversely affect metabolism or bioavailability of vitamin D [8, 12]. Recent studies have shown that body mass index (BMI) and body fat content are inversely related to serum 25(OH)D levels and directly related to parathyroid hormone (PTH) levels, which is likely due to vitamin D sequestration in body fat compartments [13, 14]. Dietary sources of vitamin D are limited, and obtaining a sufficient amount from regular diet is often problematic for many people whose diet does not normally include that few foods that are naturally rich in vitamin D. Patients with fat

malabsorption syndrome are at especially high risk of vitamin D deficiency [10]. Among elderly patients, multiple factors contribute to vitamin D insufficiency, including dietary deficiencies and decreased cutaneous synthesis due to reduced ability of the skin to synthesis vitamin D_3. A 70-year-old produces approximately 4 times less vitamin D via cutaneous synthesis compared with a 20-year-old. [15, 16]

Serum concentration of 25(OH)D is the best indicator of vitamin D status. It reflects vitamin D produced cutaneously and that obtained from food and supplements and has a fairly long circulating half-life of 15 days. Serum 25(OH)D levels do not indicate the amount of vitamin D stored in body tissues. Although 25(OH)D requires additional hydroxylation in the kidney to become active as $1,25(OH)_2D$, serum concentrations of $1,25(OH)_2D$ should never be used to determine vitamin D status. The reasons for this are that the half-life of $1,25(OH)_2D$ in the circulation is <4h, its concentrations are ~1000-fold less than those of 25(OH)D, and most importantly, as a person becomes vitamin D deficient, there is a compensatory increase in PTH secretion, which stimulates the kidney to produce more $1,25(OH)_2D$. As a person becomes vitamin D deficient and 25(OH)D concentrations decrease, $1,25(OH)_2D$ concentrations are maintained in the normal range and sometimes are even elevated. Therefore, $1,25(OH)_2D$ concentrations are not useful and can mislead physicians into thinking that patients are vitamin D sufficient when they can be severely vitamin D deficient. [17-19] Testing of serum 25(OH)D is most useful in patients who are at a risk of vitamin D deficiency, including elderly patients, infirm patients, children and adults with increased skin pigmentation, patients with fat malabsorption syndrome, and patients with osteoporosis. Therefore, in the present study we use serum 25(OH)D as our assessment of vitamin D status. It is generally accepted that a blood level of 25(OH) D <20ng/ml is

considered to be vitamin D deficient. It is based in those adults who had a blood level above 20ng/ml and who received the 50,000 IU of the vitamin D/day had no change in their PTH level [20]. Furthermore, to maximize the efficiency of intestinal calcium absorption, it was reported that in the same women who had a blood level of 20ng/ml and was raised to on average 32ng/ml, the efficiency of intestinal calcium absorption increased by 65% [21]. Thus, to maximize effect of vitamin D on calcium metabolism, it is now recommended that a 25(OH) D be above 30 ng/ml. Based on these observations, it has been suggested that vitamin D deficiency be defined as a 25(OH)D <20ng/ml, insufficiency 20 to 29.9ng/ml, and sufficiency >30ng/ml [3] (Supplemental figure 2).

Vitamin D deficiency constitutes a largely unrecognized epidemic in many populations worldwide. It has been reported in healthy children [22, 23], young adults [24, 25], and middle-aged and elderly adults [26-28]. Typically, the prevalence of low 25(OH)D levels (<20ng/ml) is approximately 36% in healthy young adults aged 18 to 29 years [25], 42% in black women aged 15 to 49 years [29], 41% in outpatients aged 49 to 83 years [20], up to 57% in general medicine inpatients in the United States [30], and even higher in Europe (28%-100% of healthy and 70%-100% of hospitalized adults) [31, 32]. Vitamin D deficiency is also common among nonwhite populations and minimal exposure to sunlight. A study of Asian adults in the United Kingdom showed that 82% had 25(OH)D levels less than 12ng/ml during the summer season, with the proportion increasing to 94% during the winter months [33]. According to the limited researches, there is also a high prevalence deficiency in Japan. A cross sectional clinic-based study of 197 normal subjects (20-68 years) reported that the prevalence of deficiency of serum 25(OH)D (<20ng/ml) was 86.7% in March, and 33.4% in June,

respectively [34]. Okazaki et al also observed that 76% had serum 25(OH)D levels less than 20ng/ml in 107 ambulatory subjects (25-85 years) [35].

Chronic vitamin D deficiency in adults can results in secondary hyperparathyroidism, increased bone turnover, enhanced bone loss, increased risk of fragility fracture, and hypocalcemic tetany [36-38]. The increase in PTH-mediated osteoclastogenesis results in increased numbers and activity of osteoclasts. The osteoclasts resorb bone via enzymatic degradation of the collagen matrix and secretion of hydrochloric acid, releasing calcium and phosphorus into the extracellular space. The result is increased skeletal porosity, defective bone mineralization, decreased bone mineral density (BMD), osteoporosis, and increased fragility-fracture risk [38, 39]. When 25(OH)D levels are less than approximately 10ng/ml (25nmol/l), osteomalacia is usually present [40, 41]. Some studies suggest that serum 25(OH)D levels greater than 30ng/ml(75nmol/l) may be required to maximize intestinal calcium absorption and prevent secondary hyperparathyroidism-induced skeletal conditions [39, 42, 43]. (Supplemental figure3)

The VDR has been identified in skeletal muscle tissue [44, 45], and several studies support the hypothesis that low serum 25(OH)D levels are associated with muscle weakness and falls [46-48]. In a randomized, double-blind trial, treatment with vitamin D plus calcium daily for 3 months reduced the risk of falling by 49% compared with calcium alone among elderly women in long-stay geriatric care [49]. Similarly, a study of community-dwelling elderly adults in Switzerland showed that treatment with 1, $25(OH)_2D_3$ significantly reduced the number of falls for individuals with calcium intake of more than 512mg/day [50]. These studies suggest that insufficient serum 25(OH)D may prevent fractures not only by improving calcium homeostasis but also by improving musculoskeletal function. (Supplemental figure3)

The small intestine, kidneys, and bones are the primary organs and tissues responsive to vitamin D that are involve in mineral metabolism that affects skeletal health. However, the effects of vitamin D are not limited to mineral homeostasis and the maintenance of skeletal health.

In the last few years, vitamin D has received increased attention recently for its pleiotropic actions on many chronic diseases including cancer, cardiovascular disease (CVD), autoimmune disease, diabetes, and neurologic disease [51] (Supplemental figure3). It has been reported that vitamin D regulates over 900 genes [52]. The importance of vitamin D on the regulation of cells of the immune system has gained increased appreciation over the past decade with the discovery of the VDR. Within the immune system, the VDR is found in significant concentration in the T lymphocyte and macrophage population [53]. Moreover, the enzyme responsible for the final the rate-limiting hydroxylation step in the synthesis of active vitamin D, 25(OH)D-1-α-hydroxylase, is expressed by activated macrophages, allowing these phagocytic cells to synthesize and secrete 1, $25(OH)_2D$ in a regulated fashion [54]. Additionally, the major1, $25(OH)_2D$ degrading enzyme, 24-hydroxylase, is also expressed in monocyte/macrophages [55]. All of these findings, then, suggest a paracrine role for vitamin D in the immune system [56].

When foreign substances invade the body, macrophage is the first cell to recognize and engulf foreign substances. Macrophages break down these substances and present the smaller proteins to the T lymphocytes. Dendritic cells are known as the most efficient antigen-presenting cell type with the ability to interact with T cells [57]. The T lymphocytes can be differentiate into one of several subtypes, Th1, Th2 and Th17. (Supplemental figure 4)

Systemic low level inflammation is defined as two to fourfold elevations in circulating levels of proinflammatory and anti-inflammatory cytokines, natural occurring cytokine antagonists, and acute phase proteins, as well as minor increases in counts of neutrophils and natural killer cells [58]. Although these increases are far from levels observed during acute, severe infections, systemic low-level inflammation is strongly associated with lifestyle factors such as smoking, obesity, and dietary patterns, together with increased risk of CVD, type 2 diabetes (T2D), cognitive decline, and wasting cachexia (loss of skeletal muscle cells) [59-64].

Interleukin-6 (IL-6) is a pleiotropic inflammatory cytokine. First discovered as a B-cell growth factor, it is synthesized by many cell types, including T-cells, macrophages and stromal cells. The plasma IL-6 concentration is ~1pg/ml or even lower in resting healthy subjects [65-67]. In contrast, the plasma IL-6 concentration may reach 10000pg/ml in response to severe systemic infections [68]. Less dramatic increases of plasma IL-6 are found in numerous inflammatory and infectious diseases. A pathogenic role for IL-6 in the development of the metabolic syndrome has been suggested, in part because the presence of a chronic low-level increase of plasma IL-6 (usually <10 pg/ml) is associated with obesity [69], low physical activity [70], insulin-resistance [71], T2D [72], CVD [73] and may serve as a predictor of mortality [74].

The 3 interferons, IFN-α, IFN-β and IFN-γ, share various properties. IFN-α and IFN-β are primarily antiviral agents with some immunomodulatory activity. IFN-γ is primarily an immune and inflammatory modulator with 100- to 1000-fold more activity than the other interferon [75, 76]. As well known, IFN-γ, a famous cytokine of T-helper 1 (Th1) cell, is a potent activator of macrophages, and is among the primary cytokines responsible for inducing nonspecific, cell-mediated mechanisms of host

defence [77]. IFN-γ, a pro-inflammatory cytokine, is the induction of tumor necrosis factor- alpha (TNF-α) and at least one form of nitric oxide (NO) synthase [78, 79]. Recently Rocha et al. have reported that IFN-γ increased in obese children is related to insulin resistance as well as non-alcoholic steatohepatitis, and plays an important role in the progression of atherosclerotic plaque [80, 81].

Interleukin-17A (IL-17A) is a recently discovered cytokine produced primarily in T-helper 17 (Th17) cell which plays an important role in a diverse autoimmune and inflammatory diseases [82]. Conversely, IL-17A is essential for host defence against many microbes, particular extracellular bacteria and fungi. Recent studies with animal models have suggested that IL-17A is associated with metabolic diseases like diabetes and obesity [83] (Supplemental figure 5).

Evidence is accumulating that low levels of circulating 25(OH)D have been further associated with metabolic syndrome, various types of cancer and CVD. In 2006, a double-blind, randomized, placebo-controlled trial showed that vitamin D supplementation improved cytokine profiles in patients with congestive heart failure [84]. Moreover, in severe obese adults (BMI: 43.6 kg/m^2) white Europeans, serum 25(OH)D was inversely related to significant levels of CRP, IL-6 and TNF-α after accounting for age, gender, season, BMI and fat [85].

Several provocative reports have been published that also supported a role for vitamin D in reducing the risk of certain autoimmune diseases. For example, administration of calcitrio strongly reduced proinflammatory cytokine production including IFN-γ, IL-17 and IL-6 in experimental autoimmune uveitis induced mice [86]. Furthermore, in 2010, presence of 1, $25(OH)_2D$ reduced IL-17 and IFN-r levels in stimulated peripheral blood mononuclear cells (PBMCs) in early RA patients [87]. In contrast, studies on healthy

individuals have had varying results. For example, in healthy-adults during the winter, low serum 25(OH)D concentrations corresponded with increased IFN-γ and IL-6 production [88], but was ineffective at modulating IL-6 in healthy women, age 25-82, and was found no effect on IFN-γ in non-smoking males and females between the ages of 18-45 years. [89, 90] Therefore, the published literature suggested the need for further inquiry into vitamin D status and its immune system implications. Thus one purpose of this study is to evaluate the association between serum vitamin D and inflammatory cytokines in healthy Japanese adults.

Previous studies have shown a positive association between physical activity and vitamin D status. In the early 1990, a cross sectional survey of middle-aged men in New Zealand found that men who engaged in weekly leisure-time aerobic activities had significantly increased plasma levels of 25(OH)D compared with inactive men after adjustment for weekly hours of sunshine exposure [91]. Moreover, a cross-sectional survey representative of the US civilian noninstitutionalized population (NHANES III) shows the mean level of serum 25(OH)D was higher in older people engaging in more times physical activities [92]. Finally, in a cross sectional study, a significant positive association between 25(OH)D levels and vigorous physical activity was found in adolescents aged from 14 to 18 years [93].

On the other hand, a recent number of papers have documented that self-reported physical activity is correlated inversely with systemic low-level inflammation. A high self-reported degree of physical activity is associated with attenuated circulating levels of TNF-α、IL-6, CRP, and SAA compared with those devoted to a sedentary lifestyle, independently of gender, age, smoking habits, BMI, total cholesterol, blood glucose, and blood pressure in the Greek ATTICA study [94]. Additionally, 8 weeks combined

training reduced IL-17 and IFN-γ production significantly in 100 women with multiple sclerosis in PBMC and plasma [95]. Systemic low-level inflammation is strongly associated with lifestyle factors such as smoking, obesity, and dietary patterns, together with increased risk of metabolic syndrome, CVD [96], cancer [97] and all-cause mortality [98]. Therefore, it suggests that physical activity offers protection against these diseases through attenuating circulating inflammatory markers.

Therefore, physical activity may modify the association between serum 25(OH)D and inflammatory markers. But the relationship has not been examined yet. Thus, we hypothesize that serum 25(OH)D concentrations would be inversely correlated with the circulating concentrations of inflammatory markers, and the relationship was influenced by physical activity in healthy Japanese adults. (Supplemental figure 6)

Methods

Subjects: A total of 94 adults (61 women; aged 21-69yr) participated in this study. Adults were excluded from the study if they were taking vitamin D supplement, its analogues, Ca, or any drugs that could affect bone and mineral metabolism including bisphosphonates; if adults with a history of stroke, cardiac disease, chronic renal failure. The purpose, procedures, and risks of the study were explained to each participant prior to inclusion, and all adults gave their written informed consent before participating in the study, which was approved by the Ethical Committee of Waseda University. Vigorous exercise is banned in the day before testing.

Anthropometry: Height, without shoes, was measured to the nearest millimeter using a stadiometer (YL-65, Yagami Inc., Nagoya, Japan). Body mass was measured using an electronic scale with the subjects wearing light clothing and no shoes and was determined to the nearest 0.1kg (Inner Scan BC-660, Tanita Co., Tokyo, Japan). BMI

was calculated by dividing the body mass in kilograms by the square of height in metres (kg/m^2).

Body composition: Dual-energy X-ray absorptiometry (DXA) (Hologic QDT-4500, DXA Scanner, Hologic Inc., Whaltham, MA, USA), a relatively easy and non-invasive technique, was used to measure % body fat.

Blood sampling: Venous blood samples were collected after fasting for at least 12h. Serum and plasma samples were stored at -80℃ until subsequent analyses. 25(OH)D serum concentrations were measured using an enzyme-linked immunosorbent assay (Immundiagnostik AG). Plasma IL-17, IL-6, IFN-γ and serum PTH were measured using an ELISA (IL-17: GEN-PROBE, IL-6: HS600B, R&D Systems; IFN-γ: BD Biosciences; PTH: Abnova). The intra-assay and inter-assay CV were 8.9% and 10.6% for serum 25(OH)D, 11.1% and 11.8% for IL-17, 4.2% and 12.8% for IFN-γ, 7.3% and 7.8% for IL-6, 3.5% and 5.2% for PTH, respectively.

Physical activity: Physical activity (PA) was assessed objectively using the activity monitor (Kenz lifecorder Plus, Suzuken Co. Ltd) worn over ten consecutive days. Participants with fewer than three days of activity recorded were eliminated from the data analysis (2 adults). On the test day, adults were instructed how to use the instrument, and were told to wear it on their belt or waistband from the moment they got up until they went to bed except while bathing or swimming. The pedometer was firmly attached to their clothes at the waist with the aid of a clip. The adults were also instructed to conduct their lives as normally as possible while wearing the accelerometer. The recorded data were uploaded to a personal computer of analysis using dedicated software. The parameters calculated were the time (minutes) per day spent in moderate and vigorous physical activities. Daily and total movement counts per day were

converted to minutes per day spent in moderate (3-6 metabolic equivalents) and vigorous (7-9 metabolic equivalents) physical activity by software for the device.

Statistical analysis: Descriptive statistics for raw variable are expressed as mean± SD. Differences among the means for gender subgroups were tested by student's t test for all continuous independent variables. Continuous parameters with a nor-normal distribution were log-transformed prior to the all subsequent analyses. Pearson's correlation coefficients were computed between serum 25(OH)D levels and the IL-6, IFN-γ, IL-17 and PTH and resulting Pearson's coefficients were used to identify the significant covariates using multiple linear regression analysis. All statistical tests were used a significance level of 5%. Statistical analyses were performed by IBM SPSS Statistics 20 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Participant Characteristics

A total of 94 adults 21-69 years of age (61 women) participated in this cross-sectional study. Group-specific means for age, height, weight, BMI, percentage body fat, 25(OH)D, PTH, MVPA, VPA and inflammatory markers are summarized in Table 1. There were no significant differences in age, MVPA, VPA or plasma inflammatory markers between men and women. Height, weight and BMI were significantly higher in men than in the women (P <0.001). Percent body fat value was significantly lower in the men than in the women (P <0001). The mean PTH concentration in women was significantly border line higher than that in men (P=0.052) (Table 1). Furthermore, there was border line significantly inverse association between serum 25(OH)D and PTH concentrations (r=0.189; P=0.069) (Figure 3).

Serum 25(OH)D Status

Serum 25(OH)D levels were higher in men (16.5±9.7ng/ml) than in women (12.5± 6.9ng/ml) (Table1 and Figure2). The overall prevalence rates of vitamin D insufficiency (20 to 29.9 ng/ml) and deficiency (<20ng/ml) were 19% and 78%, respectively (Figure1). Vitamin D insufficiency rates were observed to be 14.8% and 27% in women and men, respectively. Vitamin D deficiency rates were 83.6% and 67% in women and men, respectively (Figure 2).

Inflammatory marker outcomes

Mean plasma IL-6, IL-17, IFN-γ did not significantly differ between genders (Table 1). Serum 25(OH)D levels were not associated with plasma inflammatory markers in 94 adults (Figure 4). No significant relationship was found between serum 25(OH)D and inflammatory markers in women and men respectively, expect plasma logIL-17 in men (Figure 5). Multivariate linear regression analyses were conducted to examine the independent associations of serum 25(OH)D and logIL-17 in men (Table 2). Serum 25(OH)D was positively related to significant level of logIL-17 even after adjusted by age and percentage body fat. But the relationship was disappeared when vigorous physical activity was adjusted additionally.

Table 1 Subject characteristics divided by gender

Variable	Total	Men	Women	P Value*
Age (yr)	44 ± 14	43 ± 16	44 ± 13	0.563
Height (cm)	160.8 ± 17.0	170.1 ± 5.7	155.8 ± 18.9	<0.001
Weight (kg)	58.6 ± 10.4	69.1 ± 6.5	53.0 ± 7.3	<0.001
BMI (kg/m^2)	22.2 ± 3.0	24.0 ± 2.6	21.2 ± 2.8	<0.001
Body fat (%)	23.5 ± 6.3	18.1 ± 4.8	26.5 ± 5.0	<0.001
25(OH)D (ng/ml)	13.9 ± 8.1	16.5 ± 9.7	12.5 ± 6.9	0.041
IL-6 (pg/ml)	0.47 ± 0.53	0.43 ± 0.43	0.49 ± 0.58	0.900
IFN-γ (pg/ml)	1.02 ± 0.89	1.14 ± 0.90	0.95 ± 0.89	0.300
IL-17 (pg/ml)	23.0 ± 29.24	21.17 ± 19.39	23.98 ± 33.26	0.808
PTH (pg/ml)	63.2 ± 21.7	58.1 ± 21.6	66.0 ± 21.5	0.052
MVPA (min/d)	33.6 ± 21.2	34.3 ± 23.9	33.1 ± 19.8	0.913
VPA (min/d)	4.0 ± 5.2	4.8 ± 6.9	3.5 ± 4.0	0.784

Data are means±SD. PTH, Parathyroid hormone; MVPA, Moderate to vigorous physical activity; VPA, Vigorous physical activity;

25(OH)D, 25-hydroxyvitamin D; IFN, interferon; IL, interleukin.

*For gender difference.

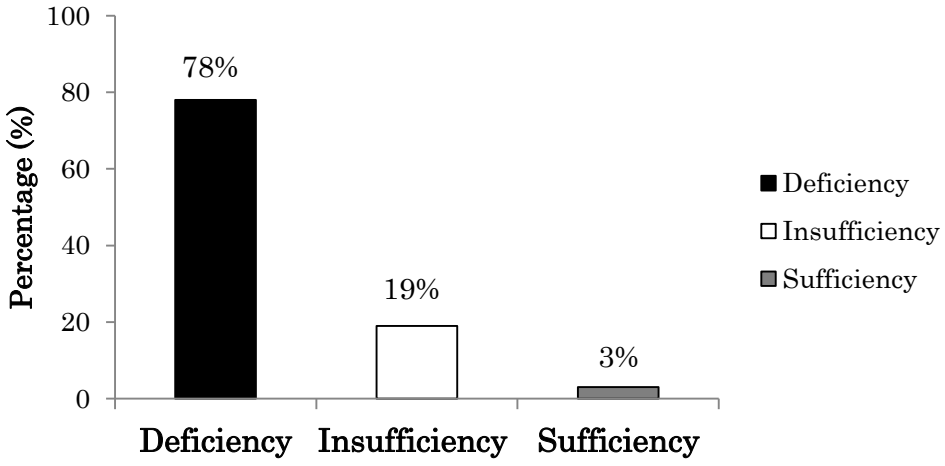


Figure1. High prevalence of vitamin D insufficiency and deficiency in 94 health Japanese

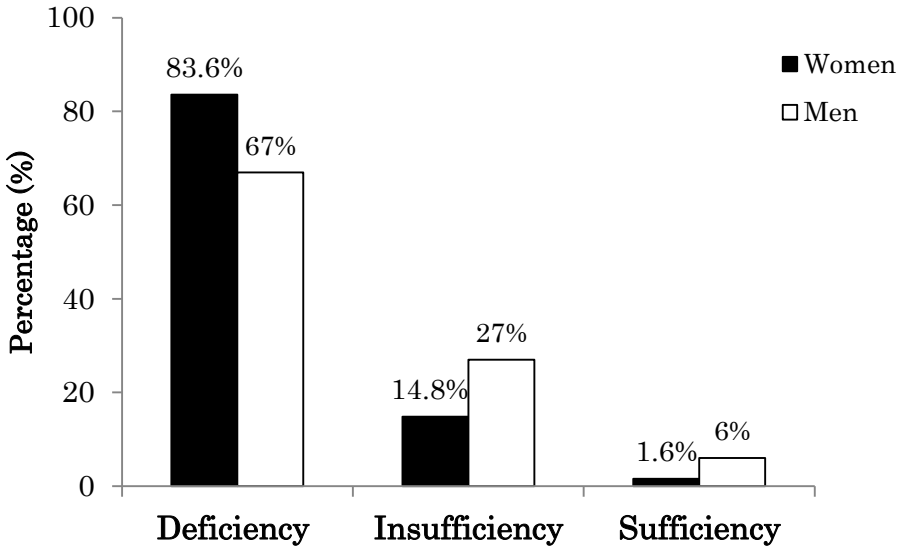
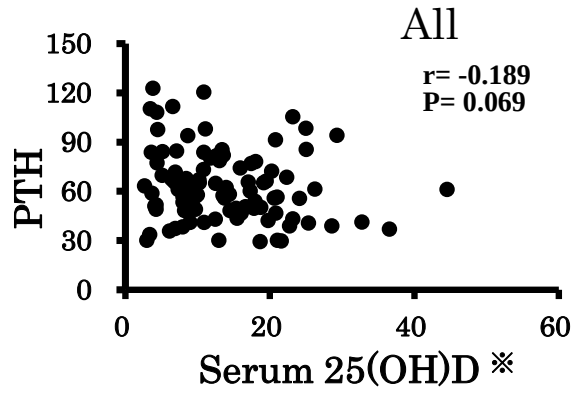
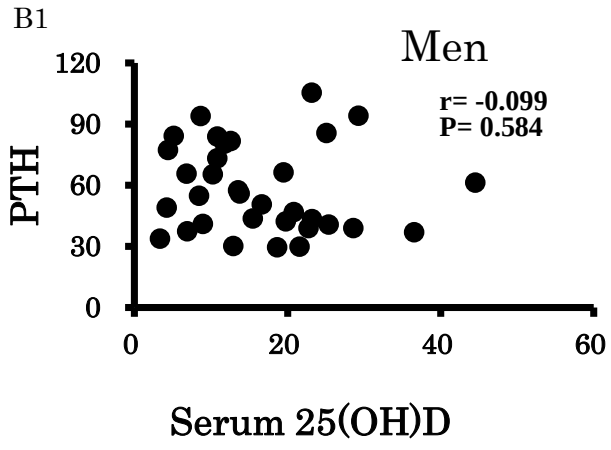


Figure2. High prevalence of vitamin D insufficiency and deficiency in women and men





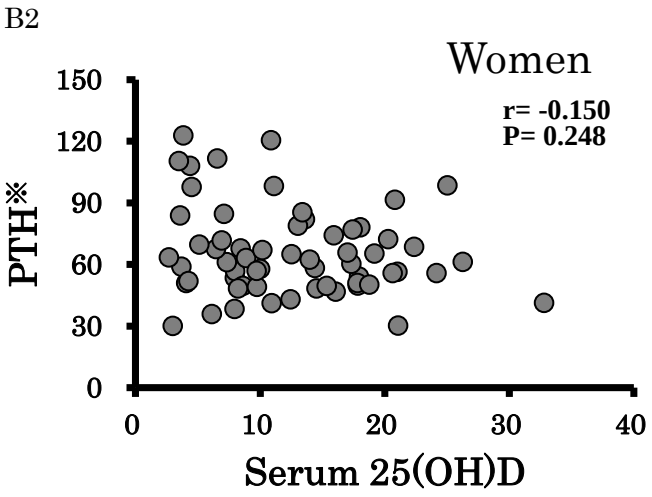
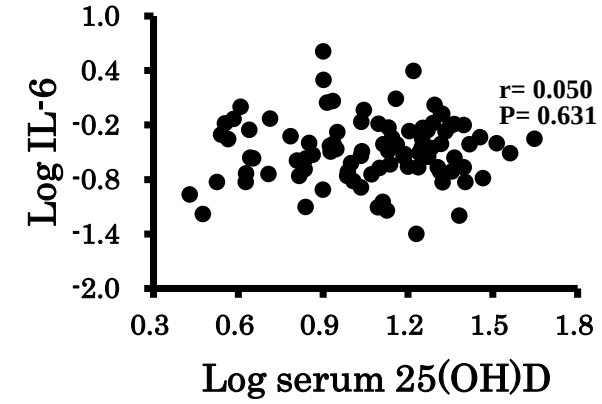
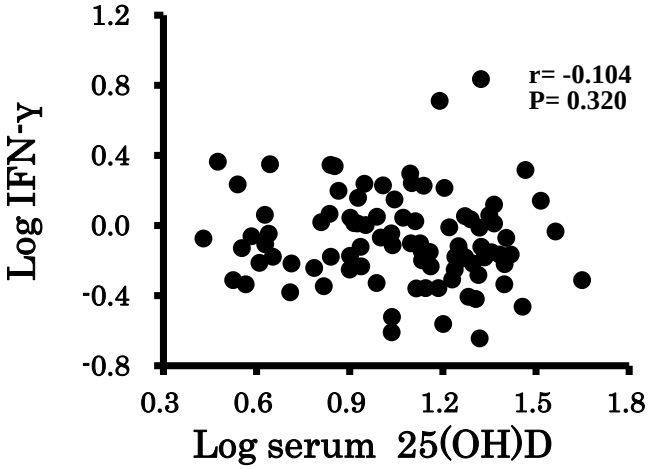


Figure 3. Relationships between serum 25(OH)D and logPTH in all 94 adults , men and women. ※log-transformed before performing the analysis.





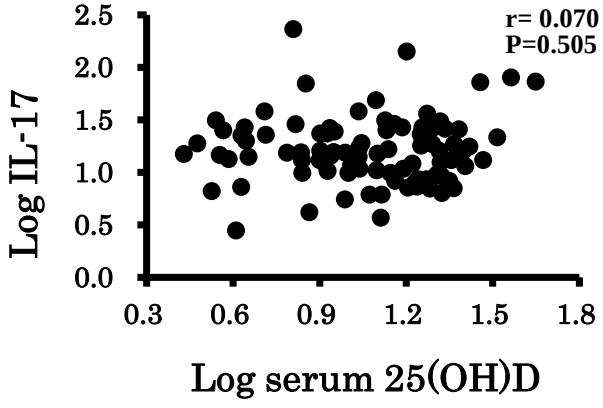


Figure 4. Relationships between serum log25(OH)D and plasma concentration logIL-6, logIFN-γ and logIL-17 in 94 adults.

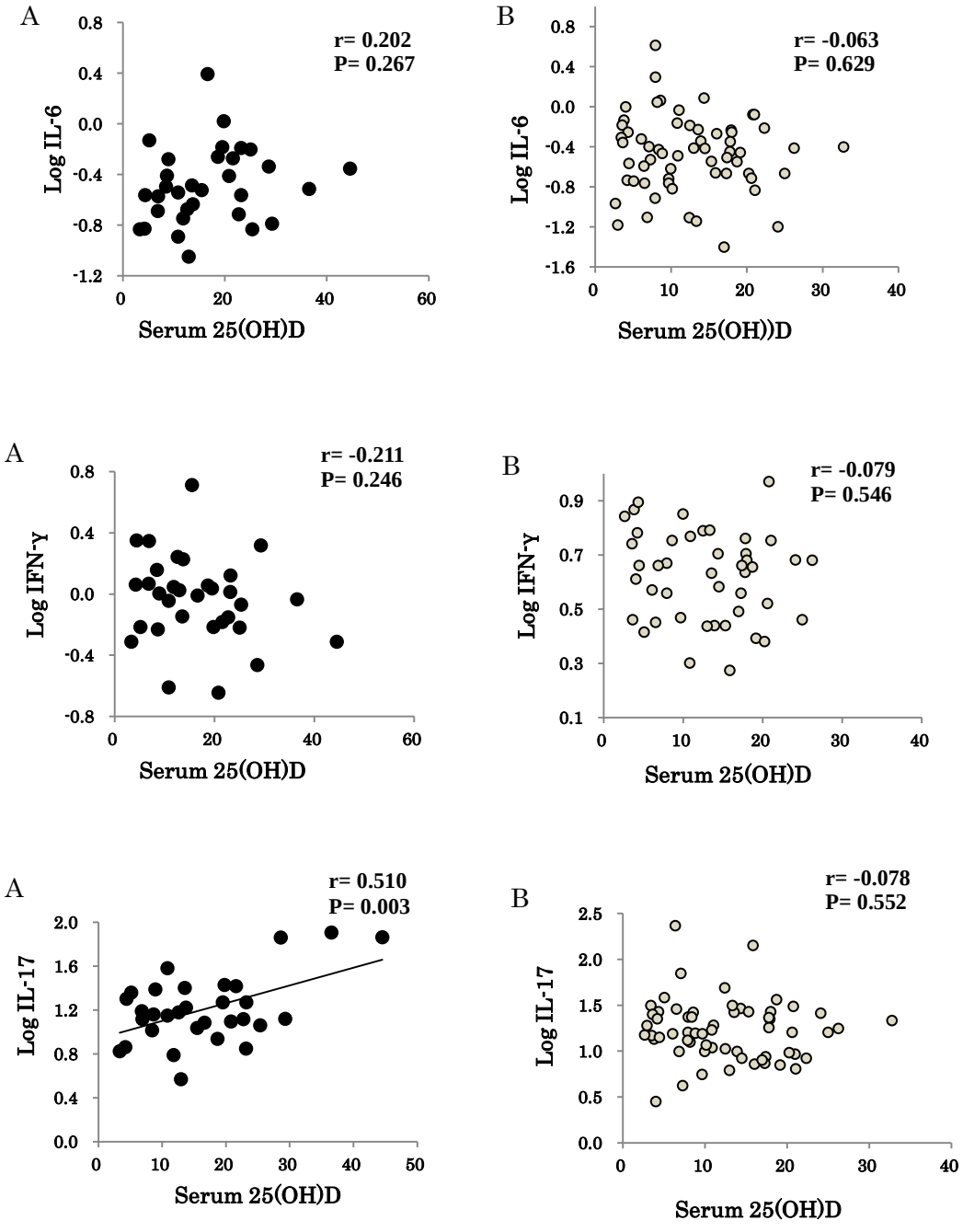


Figure 5. Relationship between serum 25(OH)D and plasma concentration of inflammatory markers in men(A) and women(B), respectively.

Table 2. Results from multiple linear regression analysis examining the association of serum 25(OH)D with plasma IL-17 in men.

	β	P	R^2
Model 1			
Serum 25(OH)D (ng/ml)	0.451	**0.018**	0.277
Model 2			
Serum 25(OH)D (ng/ml)	0.392	**0.04**	0.249
Model 3			
Serum 25(OH)D (ng/ml)	0.303	0.145	0.274

Model 1was adjusted for age and percentage body fat; Model 2 included model 1 plus moderate to vigorous physical activity (MVPA); Model 3 included model 1 plus Vigorous physical activity (VPA). MVPA, VPA and plasma IL-17 were log-transformed before performing the analysis

Discussion

This cross sectional study was performed to examine whether serum 25(OH)D is associated with inflammatory markers in healthy Japanese men and women aged 20-69yr. The main findings of the present study were as follows. First, the present study highlighted a high prevalence of serum 25(OH)D insufficiency and deficiency in men and women. Second, elevated serum 25(OH)D concentrations may be associated with increased plasma IL-17 concentrations in men, but not in women. Finally, the relationship between 25(OH)D and plasma IL-17 was found to be influenced by vigorous physical activity in healthy men. The data firstly indicate that subtle fluctuations in serum 25(OH)D concentrations modulate a Th17 type cytokine in healthy men, and the relationship may be influence by the intensity of physical activity.

The present study is one of the few studies investigating vitamin D status in the healthy Japanese in the Spring. Our data demonstrate that low vitamin D status is common among Japanese adults. Vitamin D insufficient constitutes a largely unrecognized epidemic not only in United States and Europe, but also in Asia. In India 96% of neonates [99], 91% of healthy school girls [100], 78% of healthy hospital staff [101], and 84% of pregnant women [99] were found to have vitamin D deficiency (<20ng/ml). Moreover, prevalence of serum 25(OH)D insufficiency (<30 ng/ml) was 38% in Bangladeshi, 47% in Thailand, 49% in Malaysia, and 92% in South Korea [102, 103]. In a study from Beijing, 89% of Chinese adolescent girls had serum 25(OH)D deficiency [104], and 48% of old men and 29% of young men had severe serum 25(OH)D deficiency (<10ng/ml) [105]. In Japan, studies of serum 25(OH)D were mainly concerned with in elderly and in women (Supplemental table 4). A large multi-national study found that 90% of the 198 postmenopausal Japanese women with

osteoporosis (aged 41-96yr) had 25(OH)D levels <30ng/ml [106]. An inactive lifestyle and low fish consumption were predictors of low 25(OH)D levels in a study of 263 frail female and male elderly (mean age 82.9 years) requiring home care in whom there was a 15-20% prevalence of 25(OH)D levels <12ng/ml[107]. Overall, the vitamin D status in Japan is relatively better in the regions in South Asia and positively related to fish consumption, but there is still a high percentage of vitamin D insufficiency. Moreover, data collection from youth and men was scant in Japan. In the present study, we observed a high prevalence of low 25(OH)D levels in women and men, aged 20-69 years in Spring. According to the proposed definitions of vitamin D deficiency (≦ 20ng/ml) and insufficiency (≦30ng/ml), 78% of the adults were identified as having vitamin D deficiency and only 3% as having vitamin D sufficiency. The high prevalence of vitamin D deficiency was found in our research. It suggests low vitamin D status is a growing national problem in Japan.

The values of serum 25(OH)D found in women were significantly lower than men (P=0.04) in the present study, consistent with other studies [34, 92, 108, 109]. The difference could be potentially explained by the significantly higher percentage body fat in women than men, due to less sequestering to adipose tissue. Previous study hypothesized that vitamin D after absorption is sequestered and stored in fat tissues [110], and then released slowly into the circulation in which it is used biologically. Later similar evidences in animal models and other individuals confirming that adipose tissue are the major storage site for vitamin D [111-113].

A novelty of the present investigation was the significantly positive relationship between serum 25(OH)D and plasma IL-17 concentrations in healthy men, but not in women. The relationship remained significant after controlling for potential covariates

such as age, percentage body fat, but it disappeared when VPA was adjusted additionally. Gender difference of the relationship may be causally related to sex differences in percentage body fat and systemic sex hormone concentrations [114-117].

However, the result in healthy men is potentially conflict with the previous study in mice or patients, which have shown a negative relationship between IL-17 and vitamin D [118-121]. It is possible that the effect of vitamin D on IL-17 is inversely different when the immune system is stimulated, for example when subjects had inflammatory or autoimmune disease. IL-17 is a newly describe proinflammatory cytokine secreted by a subtype of T helper lymphocytes. Higher concentrations of IL-17 could be beneficial within a certain range. It is well known that IL-17 could protect against a variety of viruses and bacterial infections [122, 123]. IL-17 has been evaluated the role that it plays in the immunopathology of influenza infection through several ways including inducting of several antimicrobial peptides and neutrophil response [124]. On the other hand it has been observed that there is an inversely association between upper respiratory tract infection and serum 25(OH) D levels. The potential mechanism for it is suggested by evidence that vitamin D plays an important role in innate immunity, particularly through the antimicrobial peptide cathelicidin [125]. Recent prospective cohort [126] and case-control studies [127,128] have demonstrated a consistent association between low serum 25(OH)D levels and respiratory tract infection. Therefore, serum 25(OH)D concentrations may influence the respiratory tract infection through IL-17. Maybe it could be one explanation for the positive relationship between serum 25(OH)D and IL-17 in healthy men. (Supplemental figure 7)

Another unique finding in the present study is that VPA could modify the relationship between serum 25(OH)D and plasma IL-17 concentrations in healthy men. In the

present study, there is a strong association between VPA and serum 25(OH)D (P =0.012, R=0.439). Maybe this could explain why the relationship between IL-17 and serum 25(OH)D disappeared when VPA was adjusted additionally.

Of particular concern in the present study is the positive relationship between plasma IL-17 and serum 25(OH)D in men may be due to three men' high serum 25(OH)D concentrations. However, the three men have relevantly higher circulating IL-17 concentrations. Future studies include a larger sample size, especially those who have higher serum 25(OH)D concentrations are obviously needed to confirm this phenomenon.

Serum 25(OH)D and plasma IFN-γ were not correlated in the present study. As a non-specific inflammatory marker of general wellness, IFN-γ increased with obese [81], and diseases such as proatherogenic or liver injury [129, 130]. Moreover, in healthy-adults during the winter, low serum 25(OH)D concentrations corresponded with increased IFN-γ in PBMC through lipopolysaccharide stimulation [88]. Nevertheless, intervention study of overweight subjects (BMI >34.3kg/m^2) and healthy young adults failed to see changes in IFN-γ concentrations after a vitamin D supplementation intervention [131, 132].

Similarly, we found no statistical relationship between serum 25(OH)D and Plasma IL-6. Several in vitro studies have shown that 1,25$(OH)_2$D is capable of inhibiting the production of IL-6 in various cell types [133-136]; while most published in vivo studies have failed to show an effect of vitamin D status on circulating IL-6 concentrations in young adults [137, 138]. Moreover, in a 22 weeks intervention during winter, serum 25(OH)D concentrations were not negatively correlated with IL-6 concentrations in the young cohort, but in the older cohort (aged ≧64y) the relationship was found [139].

Several studies show circulating IL-6 concentrations increase with advancing age [116, 140]. Further, IL-6 has been implicated in age-associated diseases and frailty; and it is postulated that certain clinically important late-life changes are due to an inappropriate presence of IL-6. In the present study age was significantly related with IL-6 ($P < 0.001$), which is agreed with the previous studies , but only 8.5% of our subjects aged $\geqq$64y. Probably because the lowering of circulating IL-6, it is failed to see the influence of serum 25(OH)D on IL-6. The present study has several limitations. First, only two adults in present study are obese (BMI >30kg/m^2). Therefore our results do not necessarily apply to an obese population. Second future, a larger sample size studies investigating the relationship between serum 25(OH)D and inflammatory markers are encouraged. Third, the study is the use of a cross sectional study design, longitudinal studies are required for the causal relationship.

Conclusion

In summary, this study revealed a high prevalence of serum 25(OH)D insufficiency and deficiency, and that elevated serum 25(OH)D concentrations were associated with increased IL-17 concentrations only in healthy Japanese men. In addition, the relationship was found to be influenced by the level of VPA.

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).

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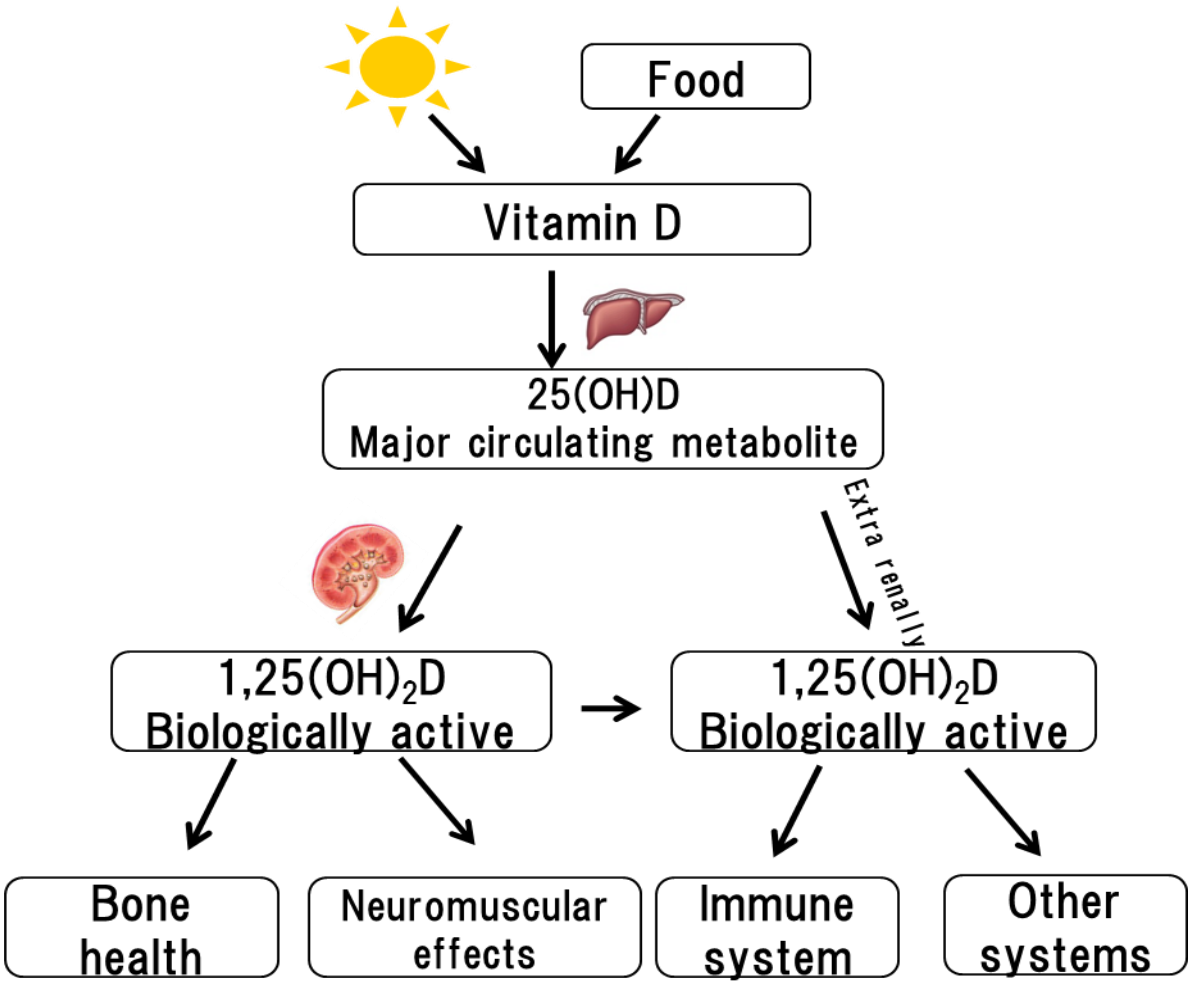
Supplemental Figures and Tables

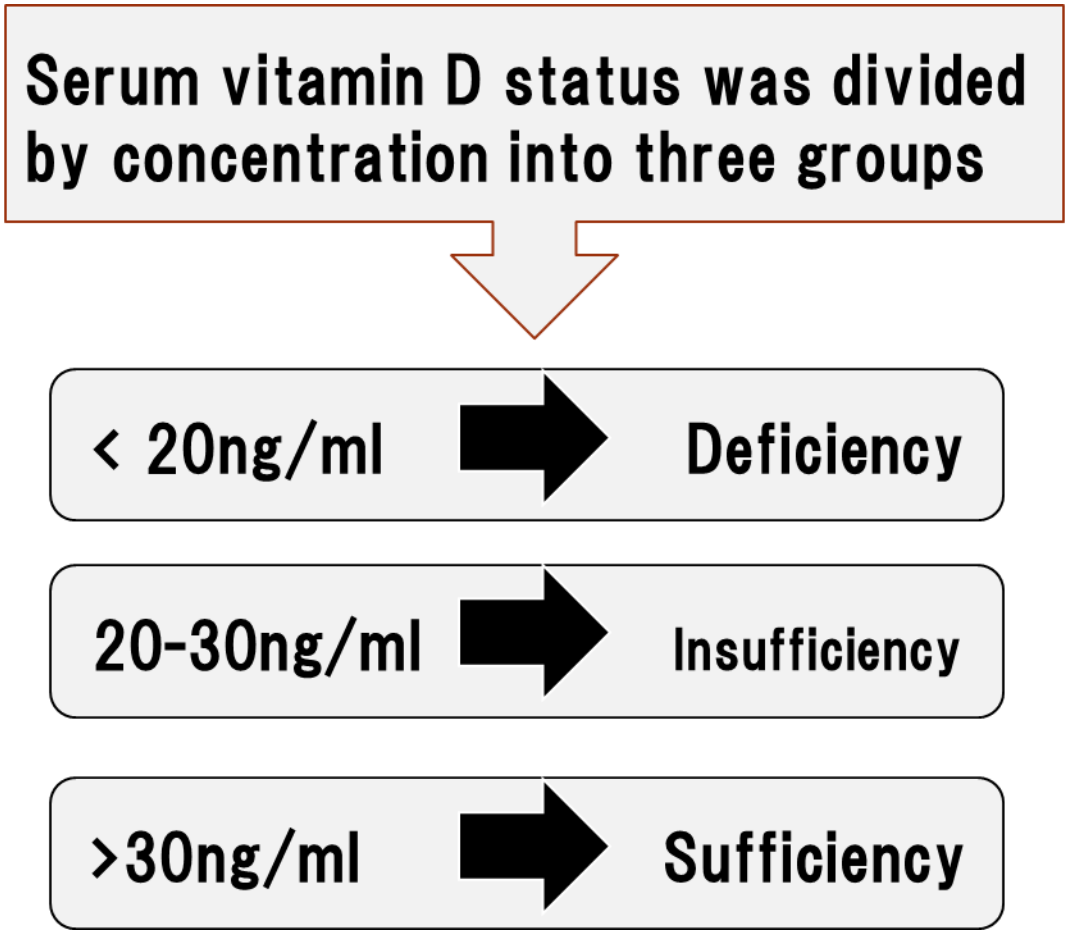


Supplemental figure 1. Production of Vitamin D and Its Metabolism and Biological Functions

Source	Vitamin D Content
Natural sources	
Salmon	
Fresh, wild (3.5 oz)	About 600–1000 IU of vitamin D_3
Fresh, farmed (3.5 oz)	About 100–250 IU of vitamin D_3 or D_2
Canned (3.5 oz)	About 300–600 IU of vitamin D_3
Sardines, canned (3.5 oz)	About 300 IU of vitamin D_3
Mackerel, canned (3.5 oz)	About 250 IU of vitamin D_3
Tuna, canned (3.6 oz)	About 230 IU of vitamin D_3
Cod liver oil (1 tsp)	About 400–1000 IU of vitamin D_3
Shiitake mushrooms	
Fresh (3.5 oz)	About 100 IU of vitamin D_2
Sun-dried (3.5 oz)	About 1600 IU of vitamin D_2
Egg yolk	About 20 IU of vitamin D_3 or D_2
Exposure to sunlight, ultraviolet B radiation (0.5 minimal erythemal dose)†	About 3000 IU of vitamin D_3
Fortified foods	
Fortified milk	About 100 IU/8 oz, usually vitamin D_3
Fortified orange juice	About 100 IU/8 oz vitamin D_3
Infant formulas	About 100 IU/8 oz vitamin D_3
Fortified yogurts	About 100 IU/8 oz, usually vitamin D_3
Fortified butter	About 50 IU/3.5 oz, usually vitamin D_3
Fortified margarine	About 430 IU/3.5 oz, usually vitamin D_3
Fortified cheeses	About 100 IU/3 oz, usually vitamin D_3
Fortified breakfast cereals	About 100 IU/serving, usually vitamin D_3
Supplements	
Prescription	
Vitamin D_2 (ergocalciferol)	50,000 IU/capsule
Drisdol (vitamin D_2) liquid supplements	8000 IU/ml
Over the counter	
Multivitamin	400 IU vitamin D, D_2, or D_3‡
Vitamin D_3	400, 800, 1000, and 2000 IU

Supplemental table 1. Dietary, Supplemental, and Pharmaceutical Sources of Vitamin D_2 and D_3 (From Holick MF. 2007)

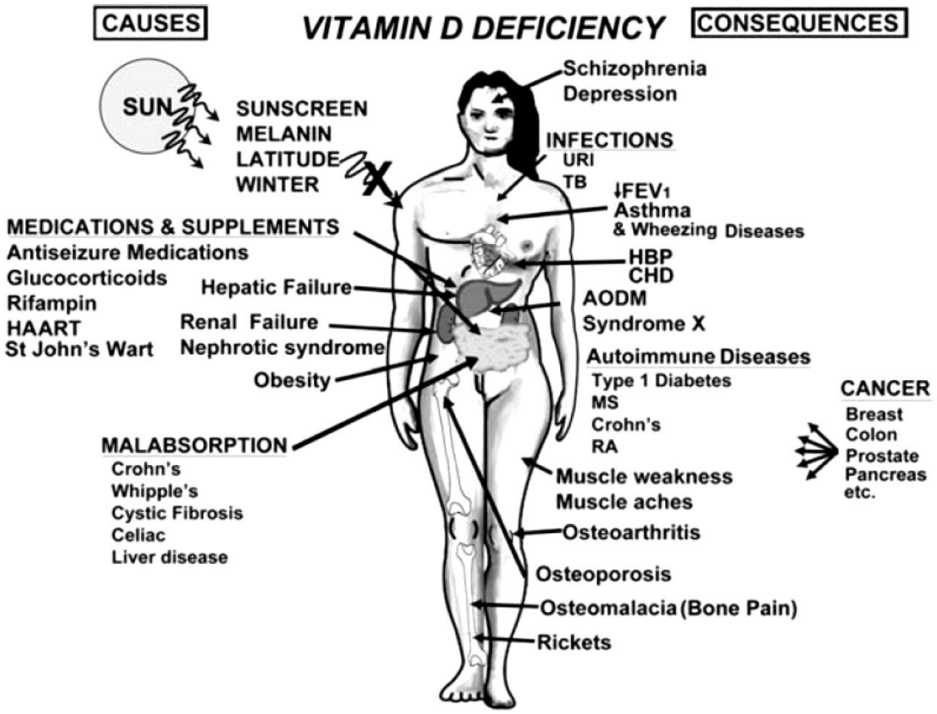
IU denotes international unit, which equals 25 ng. When the term used on the product label is vitamin D or calciferol, the product usually contains vitamin D_2; cholecalciferol or vitamin D_3 indicates that the product contains vitamin D_3; oz, ounces; a unit of weight equal to 28.34 grams. tsp, teaspoon, is a unit of volume; in U.S. , it is defined as precisely 5 ml.



Supplemental figure 2. Proposed Vitamin D Status Classification

Serum 25(OH)D ng/ml	*0*	*10*	*20*	*30*	*40*	*50*	*60*	*70*
Rickets			100%					
Osteomalacia			100%					
Cancers, all combined						75%		
Breast cancer				30%		50%		67%
Ovarian cancer					20%	25%		
Colon cancer				50%		67%		
Non-Hodgkins lymphoma				25%	30%			
Kidney cancer				50%		67%		
Endometrial cancer						35%		
Type I diabetes				50%		80%		
Type 2 diabetes				50%				
Fractures, all combined				50%				
Falls, women				72%				
Multiple sclerosis					50%		66%	
Heart attack (men)				50%				
Peripheral vascular disease				80%				
Preeclampsia				50%				
Cesarean section				75%				

Supplemental table 2. Health Benefits and Disease Incidence Prevention Related to Serum 25(OH)D Level (From Holick MF. 2010)



Supplemental figure 3. A Schematic Representation of Major Causes for Vitamin D Deficiency and Potential Health Consequences (From Holick MF. 2010)

AODM: adult-onset diabetes mellitus; CHD: coronary heart disease; FEV1: forced expiratory volume in 1 second; HAART, highly active antiretroviral therapy; HBP: high blood pressure; MS: multiple sclerosis; RA: rheumatoid arthritis; TB: tuberculosis; URI: upper respiratory infection;

	IOM			
	AI [IU (μg)/day]	*UL [IU (μg)/day]*	*Reasonable Daily allowance (IU/day)*	*SUL [IU/day]*
0–6 month	200 (5)	1,000 (25)	400–1,000	2,000
6–12 months	200 (5)	1,000 (25)	400–1,000	2,000
1–18 year	200 (5)	2,000 (50)	1,000–2,000	5,000
19–50 year	200 (5)	2,000 (50)	1,500–2,000	10,000
51–70 year	400 (10)	2,000 (50)	1,500–2,000	10,000
71+ year	600 (15)	2,000 (50)	1,500–2,000	10,000
Pregnancy	200 (5)	2,000 (50)	1,500–2,000	10,000
Lactation	200 (5)	2,000 (50)	1,500–2,000 4,000–6,000 (for infant's requirement)	10,000

Supplemental table 3. Adequate intake(AI) Tolerable Upper Limit (UL) Recommendations by IOM* and Reasonable Daily Allowance and Safe Upper Levels (SUL) for Vitamin D Based on Published Literature (From Holick MF. 2010)

The Institute of Medicine (IOM*), at the request of agencies of the U.S. and Canadian governments, assembled a committee to update the DRIs for calcium and vitamin D based upon a rigorous and comprehensive review of the scientific data.

人数	年齢（歳）： 平均±標準偏差（範囲）	調査地域 （時期）	血清 25-ヒドロキシビタミン D 濃度 （nmol/L）	対応する年齢階級の女性のビタミン D 摂取量 （年齢階級：中央値、μg/日）[2]
77	19.7（19～24）	新潟（４月）	34.2±12.1	18～29 歳：　3.1
38	（19～29）	新潟（２月）	34.0±11.0 [3]	
17	（30～39）	新潟（２月）	51.1±15.8 [3]	30～49 歳：　3.2
28	44.5±5.1（30～49）	長野（__ [1]）	45.8±14.9	
9	（40～49）	新潟（９月）	76±19 [3]	
15	（40～49）	新潟（２月）	46.5±14.8 [3]	
24	（50～59）	新潟（９月）	83±22 [3]	50～69 歳：　5.7
7	（50～59）	新潟（２月）	54.7±9.4 [3]	
244	59.5±5.7（50～69）	長野（__ [1]）	50.1±13.6	
70	（60～69）	新潟（９月）	80±16 [3]	
122	65.7（45～81）	新潟（９月）	78.6±18.2	
122	65.7（45～81）	新潟（２月）	59.7±17.1	
151	66.5±6.7（46～82）	新潟（２月）	59.9±17.0	
117	66.1±6.5（46～80）	新潟（２月）	59.1±16.1	
600	63.5±5.8 [4]	新潟（11 月）	55.6±14.6	
190	76.7±5.3（70～95）	長野（__ [1]）	48.8±15.0	70 歳以上：　5.7

[1] __：期間を限定せず。
[2] 平成 17 年及び 18 年国民健康・栄養調査[55,56]。
[3] 論文中の図から推定。
[4] 55～74 歳の女性 1,310 人が調査に登録し、そのうち 600 人が最終的に調査対象となった。この集団の年齢範囲は不明。

Supplemental table 4. Serum Levels of 25(OH)D in Japanese Women (From 「Japanese Dietary Reference Intake」 2010 edition)

性別	男性			女性		
年齢	推奨量（RDA）	目安量（AI）	耐容上限量（UL）	推奨量（RDA）	目安量（AI）	耐容上限量（UL）
0～5（月）	－	2.5(5.0)[1]	25	－	2.5(5.0)[1]	25
6～11（月）	－	5.0(5.0)[1]	25	－	5.0(5.0)[1]	25
1～2（歳）	－	2.5	25	－	2.5	25
3～5（歳）	－	2.5	30	－	2.5	30
6～7（歳）	－	2.5	30	－	2.5	30
8～9（歳）	－	3.0	35	－	3.0	35
10～11（歳）	－	3.5	35	－	3.5	35
12～14（歳）	－	3.5	45	－	3.5	45
15～17（歳）	－	4.5	50	－	4.5	50
18～29（歳）	－	5.5	50	－	5.5	50
30～49（歳）	－	5.5	50	－	5.5	50
50～69（歳）	－	5.5	50	－	5.5	50
70以上（歳）	－	5.5	50	－	5.5	50
妊 婦（付加量）				－	+1.5	－
授乳婦（付加量）				－	+2.5	－

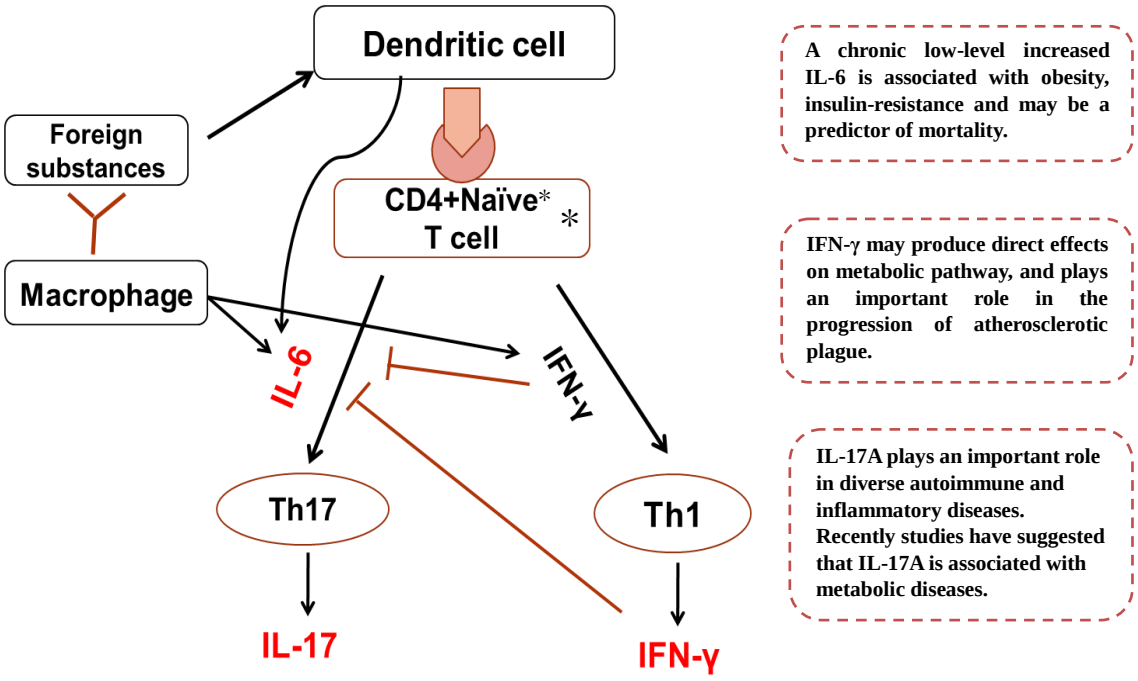
推奨量（RDA，Recommended Dietary Allowance）
ある性・年齢階級に属する人々のほとんど（97～98％）が1 日の必要量を充たすと推定される1 日の摂取量。

目安量（AI，Adequate intake）
ある性・年齢階級に属する人々が、良好な栄養状態を維持するのに十分な量。（特定の集団において不足状態を示す人がほとんど観察されない量。）

耐容上限量（UL，Tolerable Upper Intake Level）
ある性・年齢階級に属するほとんど全ての人々が、過剰摂取による健康障害を起こすことのない栄養素摂取量の最大限の量。

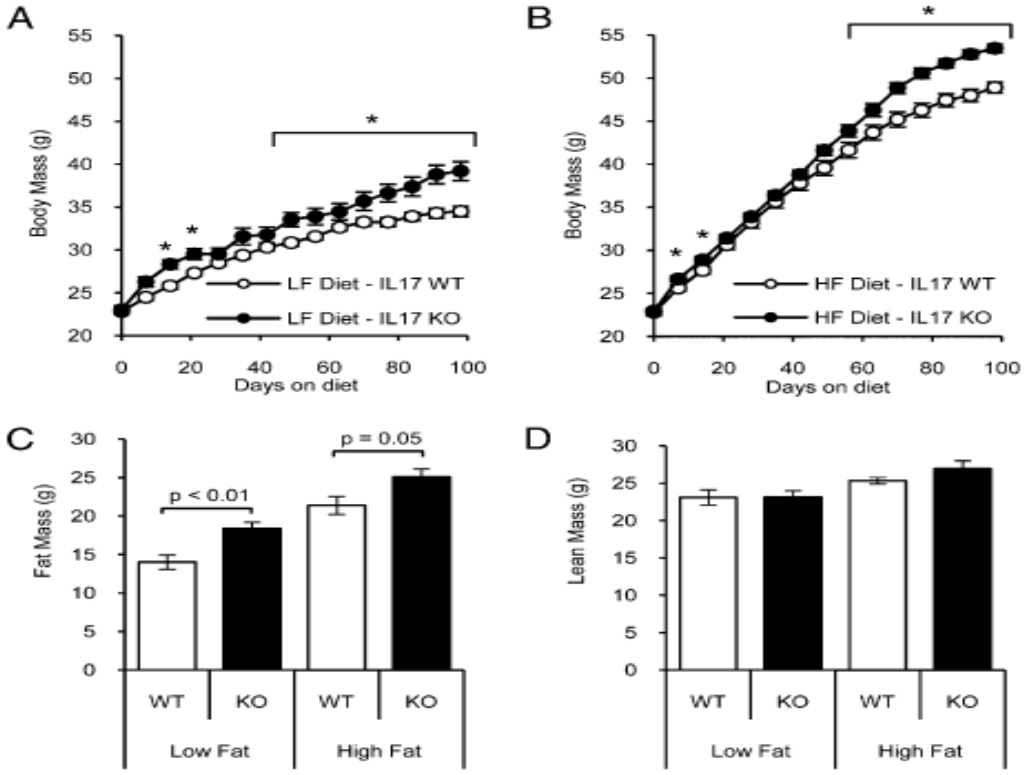
1：適度な日照を受ける環境にある乳児の目安量。（ ）内は、日照を受ける機会が少ない乳児の目安量。

Supplemental table 5. Japanese Vitamin D Reference Intake (μg/day) (From 「Japanese Dietary Reference Intake」 2010 edition)



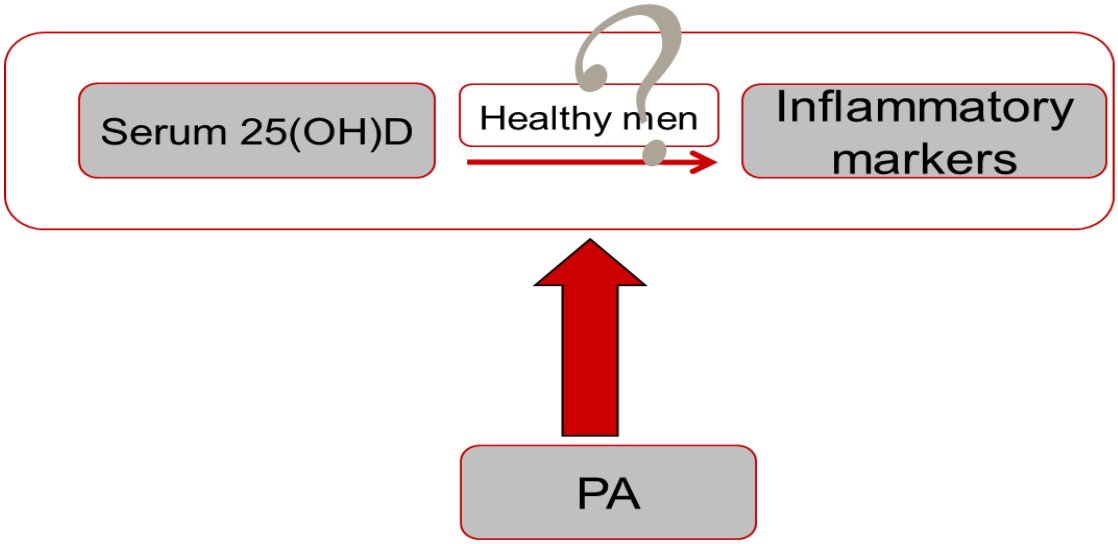
Supplemental figure 4. Interleukin-6 (IL-6), Interleukin-17 (IL-17) and Interferon-gamma (IFN-γ) Response When Foreign Substances Invade Human Body, and Their Major Functions in Immune System. Blunt-ended arrow means inhibition.

*$CD4^+$ T cells bind an epitope consisting of an antigen fragment lying in the groove of a class II histocompatibility molecule. $CD4^+$ T cells are essential for both the cell-mediated and antibody-mediated branches of the immune system. Naïve T cells are those T cells that have never been exposed to the antigen that they are programmed to respond to.



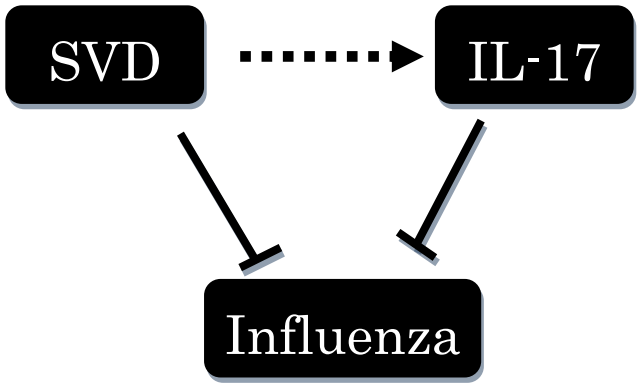
Supplemental figure 5. Enhanced Susceptibility of IL-17 Knockout (KO) Mice to Dietary Obesity. (From Jayagopi Surendar et al. 2010)

Six-to 7wk-old IL-17 wide type (WT) (n=25-30 per group) and IL-17 KO (n=14-26 per group) male mice were fed an low fat (LF) (10% fat) (A) or High fat (HF) (60% fat) (B) diet and their weights were measured over time. Data are pooled from three independent experiments, each consisting of 4-10 mice per group, and are presented as mean body mass ±SEM. ＊P <0.05 between IL-17 WT and KO groups at the indicated time points; Student t test. Mice from A and B were analyzed for body adiposity tissue (AT) mass(C) and lean mass(D) content by DEXA after 3 months of feeding.



Supplemental figure 6. Purpose of the Present Study

PA: physical activity



Supplemental figure 7. Relationship among SVD, IL-17 and Influenza

SVD: Serum vitamin D; IL-17: interleukin-17