Face page

Title: Association between vitamin D and bone mineral density in Japanese adults: The Unzen study

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Disclosure Page

Conflicts of interest

Yuzo Honda, Kazuhiko Arima, Takayuki Nishimura, Yoshihito Tomita, Satoshi Mizukami, Yasuyo Abe, Natsumi Tanaka, Michiko Kojima, Tsung-Ping Jeng, Hisashi Goto, Maiko Hasegawa, Youko Sou, Ritsu Tsujimoto, Mitsuo Kanagae, Makoto Osaki, and Kiyoshi Aoyagi declare that they have no conflict of interest.

Data accessibility statement

The datasets are available from the corresponding author in the case of reasonable request. The datasets of Unzen study analyzed in the current study are not publicly available because the datasets include in depth information and we are planning to report other association studies using the same dataset.

Authors' Roles

Study design: YH and KA. Study conduct: MO and KA. Data collection/analysis: YH, KA, TN, YT, SM, YA, NT, MK, TPJ, HG, MH, YS, RT, and MK. Statistic analysis: KA. Drafting manuscript: YH, KA, TN, TPJ, and KA. Revising manuscript content: YT, SM, YA, NT, MK, HG, MH, YS, RT, MK, MO, and KA. Approving final version of manuscript: All authors. KA takes responsibility for the integrity of the data analysis.

Mini-abstract

We showed an association between serum concentrations of vitamin D and bone health among community dwelling adults in Japan after adjustment for confounding factors, with 730 participants in a city, with concentrations of 25(OH) vitamin D and with parameters of quantitative ultrasound.

Abstract

Purpose

The primary objective of this study was to examine the correlation between serum 25-hydroxyvitamin D (25(OH)D) concentration and bone indicators as measured by quantitative ultrasound in middle-aged and older Japanese adults living in low latitude seaside areas during summer and autumn.

Methods

We conducted a cross-sectional study, The Unzen Study, on community-dwelling Japanese adults who participated to periodic health examinations between 2011 and 2013 (during the months of May to November).

Results

A total of 301 men (mean (SD) age: 67.9 (8.2) years; range: 50-92 years) and 429 women (mean (SD) age: 67.9 (7.7); range: 50-89 years) participated in this study. Serum 25(OH)D levels and quantitative ultrasound parameters (broadband ultrasound (BUA), speed of sound (SOS), and stiffness index of the calcaneus were measured for the participants. We excluded two men and 28 women from the 730 participants because they were on medication for osteoporosis. So, 299 men and 401 women were included in the final data analysis. The prevalence of vitamin D insufficiency (<30 ng/ml) was very high: 71.9% in men and 95.5% in women. In women, the log(25(OH)D) positively and significantly correlated with SOS (p=0.011) and stiffness index (p=0.028), but not with BUA (p=0.176). In men, the log(25(OH)D) did not correlate with the BUA, SOS, or stiffness index (p = 0.218, 0.420, and 0.262, respectively).

Conclusions

Serum 25(OH)D levels were associated with SOS or stiffness index in women, but not in men.

Keywords

Aging・Bone mineral density・Epidemiology・Quantitative ultrasound・Vitamin D

1. Introduction

Vitamin D is produced in the skin by exposure to ultraviolet (UV) light, or taken into the body by oral intake of vitamin D-rich food. Then, it is hydroxylated by the liver to 25(OH)D, and further hydroxylated by the kidney to 1,25(OH)$_2$D [1]. Deficiency of vitamin D results in Osteomalacia, which is characterized with the softening of the bones and with impaired bone metabolism[2]. It is thought that vitamin D would promote absorption of calcium from the small intestine and may play a partial role in calcification and osteoclast differentiation in bones. Therefore vitamin D insufficiency reduces bone strength and might be a risk factor for osteoporosis.

Previous researchers revealed that the prevalence of vitamin D inadequacy (deficiency or insufficiency) were geographically variable and ambiguous. There are several intensive researches not only among women with osteoporosis as a high-risk group, but also among population-based cohort. The prevalence of vitamin D inadequacy, defined as lower serum levels of vitamin D (<30 ng/ml), is 57.7% in European countries, 71.4% in Asian countries, 81.8% in Middle Eastern countries, 53.4% in Central and South America, and 60.3% in Australia [3]. In Japan, the prevalence of inadequacy is extremely high (82-90%) [4-6].

Body levels of vitamin D are influenced by food and UV exposure. People living in the seaside area may consume relatively high amounts of fish and foods rich in vitamin D. Low latitude areas and summertime are favorable for vitamin D sufficiency because UV light exposure is high.[7] There are no studies on the sufficiency of vitamin D in low-latitude areas during summer and autumn in Japan [4-6].

Several studies have shown a positive association between serum 25(OH)D and bone mineral density (BMD) [4, 8-12]. BMD is the main predictive risk factor for osteoporotic fracture, and quantitative ultrasound (QUS) measurements were found to be associated with increased risk of fractures [13]. The QUS measurements at the heel are an alternative investigation to BMD. This measurements are ionizing radiation-free and relatively inexpensive portable screening technique, which makes it possible to identify women at high risk of bone fragility and fracture[14] and is familiar to general practitioners in primary care [15]. However, little is known about the correlation between serum 25(OH)D and QUS parameters [15, 16]. To the best of our knowledge, there have been no reports on the correlation between serum 25(OH)D concentrations and QUS parameters in Japan.

We examined the correlation between serum 25(OH)D concentrations and bone status as measured by QUS in middle-aged and older Japanese men and women living at low latitude seaside areas in summer and autumn.

2. Materials and Methods

2.1 subjects

The participants were community-dwelling men and women aged 50 years and older residing in Unzen City, Nagasaki Prefecture, Japan. The population aged 50 years and older is approximately 13,000. Unzen City is located at (N 32 ° 50`, E 130 ° 11`) latitude and the residential area is an almost seaside area. The main industries are agriculture, fishery, and tourism. A cross-sectional study was conducted, The Unzen

Study including 730 community-dwelling adults who resident in Unzen city. Subjects were recruited from attendees who underwent an annual health examinations designed for lifestyle health check-ups and health guidance in 2011-2013 (from May to November)[17]. A total of 301 men (mean (SD) age: 67.9 (8.2) years; range: 50-92 years) and 429 women (mean (SD) age: 67.9 (7.7); range: 50-89 years) participated in this analysis. This study was approved by the Ethics Committee of the Nagasaki University Graduate School of Biomedical Sciences. All participants gave us written informed consents before the examinations.

2.2 QUS measurement

The heel QUS parameters (broadband ultrasound (BUA), speed of sound (SOS), and stiffness index) were measured using a Lunar Achilles device (Achilles InSight GE Lunar Corp., Madison, WI). The precision of this device was reported, and we evaluate it. Cepollaro et al. reported a coefficient of variation (CV) of 0.4% for SOS, 3.0% for BUA and 2.1% for Stiffness obtained with Achilles Insight [18]. We had similar precision (a coefficient of variation (CV) of 0.4% for SOS, 1.9% for BUA and 3.3% for Stiffness as intra-assay coefficient, CV of 0.3% for SOS, 0.7% for BUA and 1.7% for Stiffness as inter-assay coefficient, respectively).

2.3 Biochemical measurements

Fasting blood samples were collected, and serum 25-hydroxyvitamin D (25[OH])D) was measured by Chemiluminescent Enzyme Immunoassay. Vitamin D sufficiency was defined as serum 25(OH)D ≥30 ng/mL, vitamin D insufficiency was defined as serum 25(OH)D ≥20 ng/mL and <30 ng/mL, and vitamin D deficiency as serum 25(OH)D <20 ng/mL[19] .

2.4 Physical examination

Height (cm) and weight (kg) were obtained with light clothing and without shoes. The body mass index (BMI) was calculated as weight/height squared (kg/m^2). Information on regular exercise (a yes or no question) increased alcohol consumption (≥ 40 g/day in men and ≥ 20 g/day in women), and current smoking (yes/no) was collected by interview.

2.5 Statistical analysis

Among the 730 people, 2 men (bisphosphonate:1; active vitamin D:1) and 28 women (bisphosphonate: 20; active vitamin D: 6; SERM: 2) received medical treatment. We excluded these participants leaving 299 men and 401 women for the final data analysis. Normality was confirmed for continuous variables using Kolmogorov-Smirnov test. As 25(OH)D did not have a normal distribution, it was analyzed by performing natural log transformation. Student's *t*-test or the chi-square test was used to evaluate the differences in means of variables, and Mann-Whitney U test for the comparison of median of 25(OH)D between genders. One-way ANOVA was used to compare QUS parameters and serum 25(OH)D levels among the 10-year age groups. We evaluated the linear trend across the ranked 10-year age groups by the Jonckheere-Terpstra trend test. We applied

Pearson's product-moment correlations and multiple regression analysis adjusting for age, BMI, exercise, alcohol drinking, and current smoking to assess for correlation between the serum 25(OH)D level and QUS parameters. The data were analyzed using the SAS software package version 9.4 (SAS Institute, Cary, North Carolina). A P-value of less than 0.05 was considered significant.

3. Results

Table 1 shows the characteristics of the study population. QUS parameters (BUA, SOS, and stiffness index) were significantly higher in men than in women ($p<0.001$). Serum 25(OH)D concentrations in men were higher than those in women ($p<0.001$).

Figure 1 shows the vitamin D status (prevalence of deficiency, insufficiency, and sufficiency) among the age groups of men and women. In total, the prevalence of vitamin D deficiency and insufficiency was 15.1% and 56.9% in men, and 52.6% and 42.9% in women, respectively. The prevalence of inadequacy (deficiency and insufficiency) was higher in men than in women (71.9% in men and 95.5% in women, $p<0.001$). The prevalence of inadequacy was higher among the group of 80 years and older in both genders (90.9% in men and 100% in women) compared to the other age groups.

Table 2 shows the mean of QUS parameters (BUA, SOS, and stiffness index) and serum 25(OH)D concentrations by age group. QUS parameters significantly decreased with age in both genders. There was a weak difference between serum 25(OH)D concentrations and age groups in either gender, but not reached to a significant level ($p=0.151$ in men and $p=0.056$ in women, respectively).

Table 3 shows simple correlation coefficients between QUS parameters (BUA, SOS, and stiffness index) and age, BMI and serum log(25(OH)D). There was a negative correlation between QUS parameters and age in both genders. The log(25(OH)D) was positively correlated with SOS (p=0.012) and stiffness index (p=0.028) in women, but not in men.

Table 4 shows the Jonckheere-Terpstra trend test of vitamin D status between QUS parameters (BUA, SOS, and stiffness index). In men, vitamin D-sufficient participants (30ng/ml ≤) tended to have higher stiffness indexes (p=0.093). Women with vitamin D sufficiency showed significantly high stiffness indexes (p=0.044).

Table 5 shows the results of multiple regression analysis between log 25(OH)D and QUS parameters (BUA, SOS and stiffness index) adjusted for covariates (age, BMI, exercise, current smoking and alcohol drinking). Log 25(OH)D positively correlated with SOS (p=0.011) and stiffness index (p=0.028) in women, but not with BUA (p=0.176). Log 25(OH)D did not correlate with the BUA, SOS, or stiffness index in men (p = 0.218, 0.420 or 0.262, respectively).

4. Discussion

4.1 Vitamin D and QUS

Our study showed that serum 25(OH)D levels were positively associated with SOS and stiffness index in women, but not in men. Serum 25(OH)D levels were not associated with BUA in either men or women. Several studies have shown a positive association between serum 25(OH)D and BMD[4, 8-12]. However, the association between serum 25(OH)D levels and QUS parameters has been controversial. Serum 25(OH)D level has been reported to be an independent determinant of BUA and SOS in both men and women[16]. On the other hand, another study showed that individuals with 25(OH)D levels ≥20 ng/mL had higher SOS than those with 25(OH)D levels <20 ng/mL in a combined group of men and women [15]. And other studies were reported, which showed no association between them[20, 21]. The reason for the difference in the associations with BUA among studies is not clear. Further studies are needed to elucidate the association between serum 25(OH)D levels and each QUS parameter.

Bone is hard tissue against rapid force but is a target organ by continuous stimuli, such as aging, estrogen hormone, chronic persistent inflammation and pathological condition of kidney disease and diabetes mellitus. For a long time, small changes would be accumulated and lead to bone fragility. QUS measurements reflect qualitative properties, [22] considering that BUA is mainly influenced by the structural characteristics of trabecular bone as porosity [23, 24] and that SOS is an indicator of bone elasticity properties [25]. Our results suggest that elevated serum 25(OH)D levels contribute to increased bone elasticity in Japanese women.

4.2 Sun exposure

This study was conducted in a low-latitude area during summer and autumn in Japan. More sun exposure is thought to favor the participants because of the more production of vitamin D by UV light, instead of dietary intake. Nevertheless, the prevalence of vitamin D sufficiency (>30 ng/mL) was very low: 28.1% in men, and 4.5%

in women. Sunlight exposure is needed to improve vitamin D levels. It is thought that serum 25(OH)D concentration increases with outdoor activity (sunlight exposure)[26, 27]. The low prevalence of vitamin D sufficiency in this study may be because Japanese farmers and fishermen often wear long sleeves, hats, and gloves, and women are more likely to use sunscreen during outdoor activities. It is possible that sunlight exposure was not sufficient even in low-latitude areas and during summer and autumn.

4.3 Vitamin D intake from food

Our results showed the high prevalence of inadequacy (deficiency and insufficiency) in both gender (71.9% in men and 95.5% in women). Intake of vitamin D from food would be a promising strategy to overcome the inadequacy, as vitamin D-enriched milk or vitamin D supplements are not yet common in our country.

In a study of high-latitude European women (average age 68.4 years), the serum 25(OH)D concentration was 29.3 ng/mL and vitamin D inadequacy was 57.7% [3]. Although it is a region with limited sun exposure, it is thought that the high intake of fish rich in vitamin D and vitamin D supplementation was the reason for the relatively high serum 25(OH)D concentration [3]. Although it is desirable to regularly consume vitamin D-rich food such as salmon or shiitake mushrooms, it is expected that it would be difficult to achieve the recommended nutritional requirements on a general diet alone[3]. Vitamin D-enriched milk or vitamin D supplements are considered necessary in Japan as well as in Western countries.

4.4 Comparison among Japanese studies

In this study, the prevalence of vitamin D inadequacy was 71.9% in men and 95.5% in women. In other studies in Japan, Yoshimura et al. reported the prevalence of vitamin D inadequacy in 82.5% of men and women [5], and Tamaki et al. reported vitamin D inadequacy in 90% of women [6]. The prevalence of vitamin D inadequacy seems to be higher in Japan than in Western countries [3].

4.5 Gender difference in concentration of 25(OH)D

Our results showed a significant difference in concentration of serum 25(OH)D among genders. Higher concentration in men was consistent with previous reports (5, 7, 12, 15, 27). Factors were reported to be associated with concentration of vitamin D, such as age, BMI, education, physical activities, smoking, and drinking [5, 12]. Fat tissue and related cytokines and persistent inflammation would be one of potential candidates. Our findings obtained from a cross sectional setting could not be a clear cue to understand the mechanism, because of an absence with information about the differences in genetic factors, the amount of estrogen, or activity of adipocytokines. Further investigations, which focus on the dynamics and bioavailability of vitamin D, are warranted.

4.6 Limitations

This study has potential limitations. Because we used a cross-sectional design, we cannot establish causal relationships between serum 25(OH)D concentrations and QUS parameters. Second, there is a possibility of selection bias because our subjects were periodic health examination participants. Third, generalizations of our results to other populations should be made with caution. Forth, we could not analyze adjusting for the taking calcium or vitamin D supplements as a confounder. Fifth, we did not have information about the factors influencing the production or consumption of vitamin D. Sixth, we could not avoid a variance of season in quantification of 25(OH)D because we did this survey from May to November.

5. Conclusion

In our study, the prevalence of vitamin D sufficiency (>30 ng/mL) was very low: 28.1% in men and 4.5% in women despite the low latitude area with high sun exposure in Japan. Serum 25(OH)D levels were positively associated with SOS or stiffness index in women, but not in men. The bone health strategy for patient care must consider adequate vitamin D intake in patients, especially elderly women.

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Figure legend

Figure 1.

Vitamin D status (prevalence of deficiency, insufficiency and sufficiency) among the different age groups of the study participants. A) Vitamin D status in men, B) Vitamin D status in women.

Vitamin D status was defined as serum 25(OH)D $\geq$30 ng/mL, vitamin D insufficiency was defined as serum 25(OH)D $\geq$20 ng/mL and $<$30 ng/mL, and vitamin D deficiency as serum 25(OH)D $<$20 ng/mL. Black color indicates deficiency of vitamin D; Gray color indicates insufficiency of vitamin D; white color indicates sufficiency of vitamin D, respectively.

The prevalence of vitamin D deficiency and insufficiency was 15.1% and 56.9% in men, and 52.6% and 42.9% in women, respectively.