

Low Serum Concentrations of 25-Hydroxyvitamin D in Young Adult Japanese Women: A Cross Sectional Study

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OBJECTIVES: The vitamin D nutrition status of young adult women is unclear, but a recent preliminary report suggested that they may have vitamin D insufficiency. This study assessed the serum concentrations of 25-hydroxyvitamin D (25[OH]D), an index of vitamin D nutrition status, in young adult Japanese women in comparison with those in older women and investigated whether serum 25(OH)D concentrations are associated with other calcium-related hormones and bone mass.

METHODS: A cross sectional study of 77 healthy women, age 19 to 66 y, working in nursing homes in Japan was conducted in the winter of 1999 and 2000. The investigation included blood tests, forearm bone mass measurements, and a lifestyle questionnaire.

RESULTS: The mean serum 25(OH)D concentration in women younger than 30 y was 34.0 nmol/L (standard deviation [SD] = 11.0) and significantly lower than that in women 30 y and older (50.0 nmol/L, SD = 14.4). The proportion of subjects younger than 30 y who had serum 25(OH)D concentrations less than 30 nmol/L was 42.1% and was significantly higher ($P < 0.001$) than the proportion of those 30 y and older (10.3%). There was a weak but significant linear association between serum 25(OH)D concentrations and forearm bone mineral content ($R^2 = 0.114$, $P = 0.0052$) but not between serum 25(OH)D concentrations and bone mineral density. The association held after adjusting for body weight ($R^2 = 0.139$, $P = 0.0111$). Serum intact parathyroid hormone concentrations were within the normal range and not associated with serum 25(OH)D concentrations.

CONCLUSIONS: Serum 25(OH)D concentrations in young adult Japanese women (<30 y old) are lower than those of older adult women (30 to 66 y), and lower serum 25(OH)D concentrations are likely associated with lower forearm bone mineral content. *Nutrition* 2001;17:921–925. ©Elsevier Science Inc. 2001

KEY WORDS: bone mineral content, female, 25-hydroxyvitamin D, Japanese, vitamin D insufficiency, young adult

INTRODUCTION

Vitamin D nutrition status, which is most closely reflected by the serum concentration of 25-hydroxyvitamin D (25[OH]D), the stable form of vitamin D metabolized in the liver,¹ is a topic currently of interest in relation to bone health. Insufficient serum concentrations of 25(OH)D are thought to lead to secondary hyperparathyroidism, which is a contributing factor to the age-related acceleration of bone loss.^{2,3}

Vitamin D insufficiency is common in elderly people and seems to be a serious health problem.⁴ Vitamin D insufficiency in the elderly has been explained mainly by an age-related decrease in serum 25(OH)D concentration, probably resulting from a reduction in vitamin D biosynthesis in the skin.^{5,6} Contrary to expectations, low levels of serum 25(OH)D have been recently

reported in young women.⁷ In comparison with the large number of epidemiologic studies on the vitamin D nutrition in elderly women, however, there have been fewer studies in young women.

We reported low serum 25(OH)D concentrations in female Japanese college students,⁷ but that study was not designed to compare the serum 25(OH)D concentrations of young adult women with those of their older counterparts. Environmental factors such as seasons and occupation (indoor versus outdoor) are important determinants of serum 25(OH)D concentration because of their relation to ultraviolet exposure and may have considerably affected the results of the study. Thus, a better designed epidemiologic study was needed to confirm our preliminary findings.

To address the question of whether young adult women have inadequate vitamin D nutrition status, we studied a wide age range of adult working women in the same setting. The purposes of the present study were to assess the serum 25(OH)D concentrations of young adult Japanese women in comparison with their older counterparts and evaluate the effects of low 25(OH)D concentrations by simultaneously evaluating other calcium-regulating hormones, in particular parathyroid hormone (PTH) and 1,25-dihydroxyvitamin D (1,25[OH]₂D), and bone mass.

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SUBJECTS AND METHODS

We targeted an accessible adult female population—127 healthy women working at three nursing homes in Niigata (latitude 37°48 to 59°N), Japan—and 80 (63.0%), aged 19 to 66 y, agreed to participate in this study. Three of the women were taking vitamin D supplements and were excluded. No subject was taking any sex steroid hormones or medication for osteoporosis. Ultimately, 77 women were included in the analysis. The subjects were engaged in ordinary daytime work, but 39 subjects were also on night duty four or five times a month. The day after night duty was always a day off. Informed consent was obtained from all subjects. No foods in Japan are fortified with vitamin D. The design of this study was approved by the Ethics Committee of Niigata University School of Medicine.

The investigation was conducted in conjunction with a vitamin D nutrition study on the institutionalized elderly in the winter of 1999 to 2000 and included blood tests, bone mass measurements, and a lifestyle questionnaire. The subjects in nursing home A were examined in early December, those in home B in the late December, and those in home C in mid-February. The total hours of sunshine in the 5-wk period before each examination, which is thought to reflect current serum 25(OH)D concentrations,⁸ were 107 (3.1 h/d), 52.2 (1.5 h/d), and 61.4 (1.8 h/d), respectively.

Non-fasting blood specimens were drawn from a cubital vein and promptly stored at approximately 5°C in a refrigerator. After centrifugation, the serum was frozen the same day and maintained at -80°C until the biochemical analyses were conducted, in March 2000. Serum 25(OH)D was determined by high-performance liquid chromatography.⁹ The interassay coefficients of variation (CVs) were 4.2% for 25(OH)D₂ and 2.6% for 25(OH)D₃. We measured serum intact PTH to determine whether secondary hyperparathyroidism was present due to decreased serum 25(OH)D (25[OH]D₂ + 25[OH]D₃) concentrations. Serum intact PTH was determined by two-site immunoradiometric assay (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA).¹⁰ The reference value of intact PTH in the laboratory was 1.06 to 6.90 pmol/L, and the interassay CV was 6.5%. Serum 1,25(OH)₂D₃, to which 25(OH)D₃ is converted in the kidney and which is the final activated form of vitamin D, was determined by radioimmunoassay (IDS Ltd., Boldon, England, UK; reference value = 48 to 144 pmol/L), with an interassay CV of 14.8%. The bone mineral content (BMC) and bone mineral density (BMD) of the non-dominant forearm were measured in 74 subjects by dual-energy x-ray absorptiometry with a DTX-200 Osteometer (software version 1.54J, Osteometer MediTech A/S, Rødovre, Denmark).¹¹ The long-term CV value using the standard phantom was 0.7%.

A self-reported questionnaire on consumption of vitamin D-rich food, sunscreen use (yes or no), menstrual history, body weight, and current medication was administered to the subjects. The subjects were specifically asked to report the frequency of their consumption of vitamin D-rich foods, including fish, the major source of vitamin D in Japanese people,¹² and eggs by choosing one of the following: 0 = rarely (less than once/wk), 1 = sometimes (1 to 3 times/wk), or 2 = frequently (4 or more times/wk). The reliability of the self-reported weight of healthy Japanese women was confirmed previously.¹³

The mean and standard deviation (SD) were calculated for continuous variables. One of the aims of this study was to assess serum 25(OH)D levels in young women and compare these levels with those of older adult women. However, we do not have a clear definition for "young adult" with regard to vitamin D insufficiency. Therefore, the study population was divided into conventional 10-y age groups, and the average 25(OH)D levels of those groups were compared. Analysis of variance with Scheffé's multiple comparison test was used to test differences between any two mean values. The chi-square test was used for statistical evaluation of associations between two categorical sets of data. Simple and multiple linear regression analyses were used to evaluate the

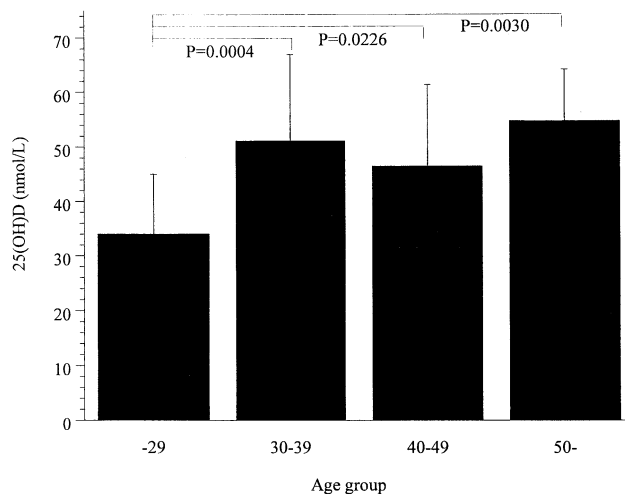


FIG. 1. The mean serum 25(OH)D concentrations of the 10-y age groups. The mean serum 25(OH)D concentrations in those younger than 30 y and those in their 30s, 40s, 50s, and older were 34.0 nmol/L (SD = 11.0), 51.1 nmol/L (SD = 15.8), 46.5 nmol/L (SD = 14.8), and 54.7 nmol/L (SD = 9.4), respectively. The mean serum 25(OH)D concentrations in those younger than 30 y was significantly lower than those in all other age groups. The mean serum 25(OH)D concentrations of those in their 30s, 40s, and 50s and older were not significantly different. 25(OH)D, 25-hydroxyvitamin D; SD, standard deviation.

associations between serum 25(OH)D concentrations and predictor variables. Multiple regression analysis also was used for the statistical adjustment of the relation between bone mass and serum 25(OH)D concentrations. Subjects on occasional night duty were coded as 1 and the others as 0. The SAS statistical package (version 6.12) was used for the computations. $P < 0.05$ was considered statistically significant.

RESULTS

The mean age of the subjects was 32.9 y (SD = 11.3), and their mean body weight was 52.0 kg (SD = 6.2). Subjects' average serum 25(OH)D₃ concentration was 42.0 nmol/L (SD = 15.1). Serum 25(OH)D₂ was detected in only three subjects (2.1, 2.4, and 3.1 nmol/L). The mean concentration of serum 25(OH)D, the sum of the serum 25(OH)D₂ and 25(OH)D₃ values, was 42.1 nmol/L (SD = 15.1). The mean serum 25(OH)D concentrations of the 10-y age groups are shown in Fig. 1. The mean serum 25(OH)D concentrations in those younger than 30 y ($n = 38$) and those in their 30s ($n = 17$), 40s ($n = 15$), and 50s and older ($n = 7$) were 34.0 nmol/L (SD = 11.0), 51.1 nmol/L (SD = 15.8), 46.5 nmol/L (SD = 14.8), and 54.7 nmol/L (SD = 9.4), respectively. The mean serum 25(OH)D concentrations in those younger than 30 y were significantly lower than those in all other age groups ($P = 0.0004$, 0.0226, and 0.0030, respectively). The mean serum 25(OH)D concentrations of those in their 30s, 40s, and 50s and older were not significantly different. In this paper, adult women younger than 30 y are referred to as "young adult women." Age was then divided into two categories: those younger than 30 y were coded as 0 and those 30 y and older as 1.

The proportions of subjects who had serum 25(OH)D concentration below 30 nmol/L, which is generally considered to represent vitamin D insufficiency,^{14,15} were 16 of 38 (42.1%) among those younger than 30 y and 4 of 39 (10.3%) among those 30 y and older, and the difference was statistically different ($\chi^2 = 10.15$, $P < 0.001$).

The lifestyle characteristics of those younger than 30 y and

TABLE I.

COMPARISONS OF THE LIFESTYLE CHARACTERISTICS OF WOMEN YOUNGER THAN 30 Y AND THOSE 30 Y AND OLDER		<30 y	≥30 y
Frequency of fish consumption	$df = 2, \chi^2 = 7.59, P = 0.022$		
Frequently, ≥4 times/wk		2 (5.4%)	11 (29.7%)
Sometimes, 1–3 times/wk		29 (78.4%)	22 (59.5%)
Rarely, <1 time/wk		6 (16.2%)	4 (10.8%)
Frequency of egg consumption	$df = 2, \chi^2 = 0.24, P = 0.885$		
Frequently, ≥4 times/wk		12 (32.4%)	11 (29.7%)
Sometimes, 1–3 times/wk		23 (62.2%)	23 (62.2%)
Rarely, <1 time/wk		2 (5.4%)	3 (8.1%)
Use of sunscreen	$df = 1, \chi^2 = 0, P = 1.000$		
Yes		15 (40.5%)	15 (40.5%)
No		22 (59.5%)	22 (59.5%)
Night duty (4 or 5 times/mo)	$df = 1, \chi^2 = 19.77, P < 0.001$		
Yes		29 (76.3%)	10 (25.6%)
No		9 (23.7%)	29 (74.4%)

those 30 y and older are compared in Table I. Subjects 30 y and older consumed fish more frequently than did those younger than 30 y, and a higher proportion of them were engaged in night duty.

Simple linear regression analysis associated serum 25(OH)D concentrations to age group (<30 versus ≥30 y old; $R^2 = 0.283, P < 0.0001$), total hours of sunshine ($R^2 = 0.218, P < 0.0001$), fish consumption ($R^2 = 0.110, P = 0.0040$), body weight ($R^2 = 0.070, P = 0.0246$), and night duty ($R^2 = 0.0671, P = 0.0229$), and multiple linear regression analysis associated serum 25(OH)D concentrations with all variables but night duty (Table II).

To evaluate the underlying adverse effects of low 25(OH)D concentration on bone health in this population, associations between serum 25(OH)D concentrations and serum intact PTH concentrations, serum 1,25(OH)₂D₃ concentrations, and forearm BMC and BMD were tested. The mean serum intact PTH and 1,25(OH)₂D₃ concentrations of the subjects were 2.88 (SD = 1.33) and 111.1 (SD = 33.6), respectively. No subject had a high serum intact PTH level, but one subject had a serum 1,25(OH)₂D₃ concentration below the reference value. Serum 25(OH)D concentrations were not significantly associated with serum intact PTH

concentrations ($P = 0.0532$), but serum 25(OH)D₃ was linearly associated with the serum 1,25(OH)₂D₃ concentrations ($R^2 = 0.064, P = 0.0267$), as shown in Fig. 2. Age and weight were not linked to serum 1,25(OH)₂D₃ concentrations. The mean forearm BMC and BMD values were 2.94 g (SD = 0.36) and 0.453 g/cm² (SD = 0.051), respectively.

To assess serum 25(OH)D concentrations in relation to forearm bone mass, the following analyses were conducted only in subjects younger than 50 y ($n = 70$) because the bone mass of women decreases rapidly thereafter. Serum 25(OH)D concentrations were linearly associated with BMC ($R^2 = 0.114, P = 0.0052$; Fig. 3) but not significantly associated with BMD ($P = 0.2597$). Univariate analysis also associated BMC to weight ($R^2 = 0.082, P = 0.0231$). Serum 25(OH)D concentration was still a significant predictor ($R^2 = 0.139, P = 0.0111$) of BMC after adjusting for weight. BMC and BMD were not significantly associated with age group, serum intact PTH, or 1,25(OH)₂D₃ concentrations.

TABLE II.

INDEPENDENT VARIABLES FOR SERUM 25-HYDROXYVITAMIN D CONCENTRATION (NMOL/L), SELECTED FROM THE SUBJECTS' AGE GROUP, TOTAL HOURS OF SUNSHINE, FISH CONSUMPTION, NIGHT DUTY, AND WEIGHT BY THE STEPWISE PROCEDURE OF MULTIPLE REGRESSION ANALYSIS				
Independent variable	Regression coefficient	SE	R ²	P
Age group*	10.5	2.9	0.272	0.0005
Total hours of sunshine	0.22	0.059	0.129	0.0004
Fish consumption†	6.7	2.5	0.044	0.0086
Weight (kg)	0.52	0.22	0.042	0.0225

* Those younger than 30 y are coded as 0, and those 30 y and older as 1.

† Frequency of fish consumption is categorized as follows: 0 = rarely (less than once/wk), 1 = sometimes (1–3 times/wk), and 2 = frequently (≥4 times/wk).

SE = standard error

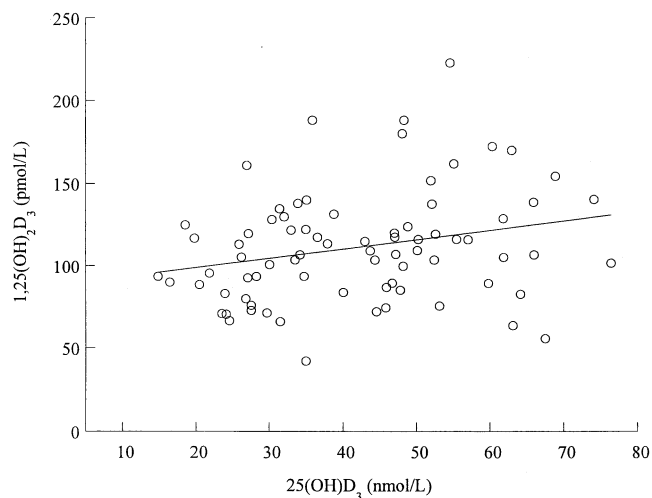


FIG. 2. Scatterplot of serum 25(OH)D₃ versus 1,25(OH)₂D₃ concentrations. The regression equation is $Y = 87.7 + 0.557X$ (Y : 1,25(OH)₂D₃ [pmol/L], X : 25(OH)D₃ [nmol/L], $R^2 = 0.063, P = 0.0280$). 1,25(OH)₂D₃, 1,25-dihydroxyvitamin D₃; 25(OH)D₃, 25-hydroxyvitamin D₃.

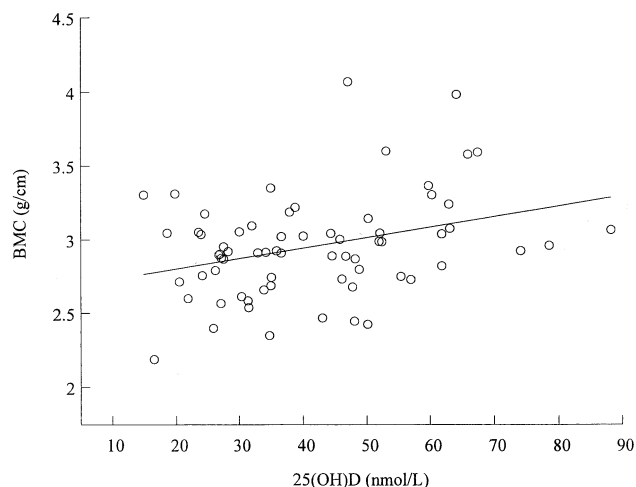


FIG. 3. Scatterplot of serum 25(OH)D concentrations versus forearm BMC (g). The regression equation is $Y = 2.64 + 0.00772X$ (Y: BMC [g], X: 25[OH]D [nmol/L], $R^2 = 0.114$, $P = 0.0052$). 25(OH)D, 25-hydroxyvitamin D; BMC bone mineral content.

DISCUSSION

The present study clearly demonstrated that the women younger than 30 y had lower serum 25(OH)D concentrations (mean = 34.0 nmol/L) than the women aged 30 to 66 y. This finding conflicts with the current belief that vitamin D nutrition status, i.e., that serum 25(OH)D concentrations decrease with age.^{5,6} Moreover, when a serum 25(OH)D concentration cutoff value of 30 nmol/L was adopted, the prevalence of vitamin D insufficiency in women in their 20s was 42.1%, which is close to the rate (40.3%) in a previous study of female junior college students.⁷ Although there have been few studies on vitamin D nutrition status in young adult women, Harris and Dawson-Hughes¹⁶ reported low 25(OH)D concentrations (mean = 30.2 nmol/L in winter) in young adult black women (20 to 40 y old).

The reason the young adult Japanese women had low serum 25(OH)D concentrations needs to be addressed. We first speculated that differences in food preferences between generations might be contributory: young people consume less fish than older people. Multivariate analysis, however, showed that young adult women's low concentration of serum 25(OH)D was independent of frequency of fish consumption. Thus, frequency of fish consumption was not a significant factor in the low serum 25(OH)D concentrations of the young adult women. We did not evaluate the actual amount of fish intake, and we still suspect that low fish intake might have been responsible for the young adult women's low serum 25(OH)D concentrations because fish consumption in winter is a crucial factor in maintaining appropriate serum 25(OH)D concentrations in Japanese women.¹² Young women's specific eating behavior in relation to dieting¹⁷ may be another contributory factor to their potential low intake of vitamin D in food.

There may have been a difference in outdoor activity between those younger than 30 y and those 30 y and older. Univariate analysis associated night duty with low serum 25(OH)D concentrations, but multivariate analysis did not. Although night duty was more prevalent in those younger than 30 y, restriction of outdoor activities by once-a-week night duty is unlikely to have greatly affected endogenous 25(OH)D₃ production. Thus, such an explanation is hard to accept because all subjects were working in a similar occupational setting. Our data did not demonstrate any importance of sunscreen use on serum 25(OH)D concentrations. In the end, we were unable to clearly identify any causal factors

responsible for the lower concentration of serum 25(OH)D in the young adult women.

Studies targeting middle-aged and elderly populations have shown positive associations between serum 25(OH)D concentrations and BMD at various bone sites.^{18–21} This relation seems to be attributable to hyperparathyroidism secondary to the low serum 25(OH)D concentrations.¹⁸ Our data showed that serum 25(OH)D concentrations in women, including young adults, were associated with forearm BMC. We recently reported that young adult women with vitamin D insufficiency had lower calcaneal bone mass as measured by quantitative ultrasound densitometry.⁷ These results suggest that vitamin D nutrition status affects the bone mass of young women. Because hyperparathyroidism was not found in these subjects, other as yet unknown mechanisms may exist. Serum 25(OH)D₃ concentrations have not been reported to have clear, direct biological effects, and thus their interactions with other calcium-regulating factors or female reproductive hormones may play a role. 1,25(OH)₂D₃ may be a clue. Our data demonstrated that serum 25(OH)D₃ concentrations reflected the serum 1,25(OH)₂D₃ concentrations, although we did not find serum 1,25(OH)₂D₃ concentrations to be associated with BMC.

The present study demonstrates that serum 25(OH)D concentrations are associated with weight-adjusted BMC, but not with BMD. An explanation for this observation is challenging. BMC is involved in the projected area; thus, BMC reflects the bone size of individuals. Low serum 25(OH)D concentrations may be correlated with bone size independent of body size. The site of measurement also might have influenced the findings. We measured BMD in the forearm, which is rich in cortical bone. Measuring BMD at this site may be less sensitive for detecting BMD differences than at other bone sites with more spongy bone. The lack of an association between serum 25(OH)D concentration and forearm BMD in an elderly Japanese women has been reported.²²

In contrast, Oliveri et al.²³ reported that peripheral bone mass is not affected by low vitamin D concentration in young adults during winter. They interpreted their findings as adequate calcium intake compensating for a vitamin D deficit. In that context, the present association between serum 25(OH)D concentrations and BMC is convincing because the calcium intake of adult Japanese women is estimated to be as low as 400 to 600 mg/d.²⁴ In any event, vitamin D is recognized as having a critically important role in the development, growth, and mineralization of the skeleton in young people,¹ and thus the effects of low serum 25(OH)D concentrations on the bone health of young adult women should be clarified.

There may be another explanation of the present results. Problem eating behaviors of young women, such as dieting,¹⁷ may lead to hypovitaminosis D, as a result of low vitamin D intake, and low bone mass, as a result of low calcium intake. This idea is supported by Van Loan and Keim's²⁵ recent finding of lower BMC values in women with high levels of cognitive eating restraint. They hypothesized that cognitive eating restraint triggers changes in women's reproductive hormones, such as lower progesterone concentrations, which in turn may cause lower BMC values.

Our study targeting women between 19 and 66 y old produced the following findings: 1) the mean serum 25(OH)D concentration of women younger than 30 y was significantly lower than those of their older counterparts; 2) the proportions of subjects who had serum 25(OH)D concentration below 30 nmol/L were 42.1% among those younger than 30 y and 10.3% in those 30 y and older, a significant difference; 3) serum 25(OH)D₃ concentrations were significantly, linearly associated with serum 1,25(OH)₂D₃ concentrations; and 4) serum 25(OH)D concentrations (in women younger than 50 y) were significantly, linearly associated with forearm BMC.

In conclusion, serum 25(OH)D concentrations in young adult Japanese women are lower than in their older counterparts (30 to 66 y), and vitamin D insufficiency seems more prevalent. In addition, lower serum 25(OH)D concentrations appear to be asso-

ciated with lower forearm BMC. The mechanisms responsible for these findings should be explored further by assessing biochemical markers of bone turnover.

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