

**ORIGINAL****High serum 25-hydroxyvitamin D levels are associated with greater lean tissue mass and skeletal muscle mass : a cross-sectional study on young healthy Japanese women**Moemi Morita<sup>1</sup>, Marino Takeuchi<sup>2,3</sup>, Masahiko Kato<sup>2</sup>, and Masae Sakuma<sup>1,2</sup><sup>1</sup>Laboratory of nutrition, Graduate school of Health, and Environmental Sciences, Fukuoka Women's University, Fukuoka, Japan, <sup>2</sup>Department of Human Nutrition, School of Life Studies, Sugiyama Jogakuen University, Aichi, Japan, <sup>3</sup>Department of Health and Nutrition, School of Health and Nutrition, Tokai Gakuen University, Aichi, Japan

**Abstract :** Studies have shown that low serum 25(OH)D levels in young women may affect not only their own health but also the health of next generation through pregnancy and childbirth. The aim of this study is to assess the serum 25(OH)D levels in young Japanese women and to determine the factors influencing the changes in their serum 25(OH)D levels. Herein, 83 healthy young Japanese women were included. Early morning fasting blood samples, habitual food intake survey, eating habits survey, and anthropometric measurements were used to analyze the relationship between the serum 25(OH)D levels and nutrient intake, intake of food groups, dietary habits, and body composition. The findings revealed that about 50% of the study participants had an adequate intake (AI) of vitamin D (8.5 µg), whereas about 88% of the study participants were vitamin D deficient and about 12% were insufficient in serum 25(OH) levels. No significant association was observed between the serum 25(OH)D levels and nutrient intake or intake of food groups. Furthermore, higher serum 25(OH)D levels were associated with higher percentages of muscle and lean tissue, indicating that regulating the serum 25(OH)D levels helps maintain muscle mass and lowers the risk of sarcopenia and locomotive syndrome. *J. Med. Invest.* 71 : 260-266, August, 2024

**Keywords :** 25- hydroxyvitamin D, young women, body composition**INTRODUCTION**

The main function of vitamin D is to regulate calcium and phosphorus metabolism, which has been conventionally known ; however, in recent years its effects on the prevention of frailty and sarcopenia have attracted attention (1). It has been reported that vitamin D receptors expressed on the skeletal muscle cells (C2C12) respond in a vitamin D-dependent manner and both 25(OH)D and 1,25-dihydroxyvitamin D (1,25-[OH]<sub>2</sub>D) regulate C2C12 growth and differentiation and increase muscle size (2). Furthermore, in an epidemiological study of Japanese women, the odds ratio of falling was significantly higher in the group with a serum 25(OH)D level of less than 20 ng/mL compared with the group with that of 25 ng/mL or higher (3, 4). Although the relationship between vitamin D and falls and the maintenance of muscle strength has not been determined, the role of vitamin D in maintaining muscle strength and preventing frailty is expected to gain more research interest in an aging society such as Japan.

The increasing prevalence of underweight among young women is also a social problem in Japan. The 2019 Japan National Health and Nutrition Survey reported that more than 20% of women in their 20s are underweight (5). Considering that excessive dietary restriction and underweight result in a loss of lean body mass, which can lead to future care needs and frailty, it is necessary to prevent poor nutrition and underweight even in young women. Moreover, it has been demonstrated

that adequate vitamin D nutritional status during pregnancy is essential for development of prenatal and postnatal children (6-8), it has been suggested that low serum 25(OH)D levels in young women may adversely affect the health of the next generation through pregnancy and childbirth. Thus, it is essential to maintain proper vitamin D nutritional status in young women. Most reports in Japan on the factors associated with vitamin D nutritional status have focused on the elderly, with only a few reports focusing on young women. Therefore, the purpose of this study is to assess the serum 25(OH)D levels of young women and to determine their association with dietary intake and body composition.

**PARTICIPANTS AND METHODS***Study participants*

This study included 83 healthy young women. Physical measurements and blood sampling were conducted, and no abnormalities were observed in any of the study participants. Table 1 shows the clinical and biological characteristics of the study participants. This study was performed after obtaining written informed consent from all the study participants and approved by the Ethics Committee of the Sugiyama Jogakuen University. The protocol was implemented in accordance with the Declaration of Helsinki.

*Protocol*

The data for this study were obtained in July. The study participants underwent four examinations : (1) blood sampling in the morning after fast (2) habitual food intake survey (brief-type self-administered diet history questionnaire [BDHQ]), (3) questionnaire survey on eating habits, and (4) anthropometric measurements. Moreover, the study participants were prohibited

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from eating or drinking anything other than water after 9 p.m. on the day before the examinations and were asked to drink water freely until the following morning.

#### Blood sampling and measurements

Venous blood samples were obtained from the study participants in a fasting state between 9 a.m. and 10 a.m. on the day of the examinations. The blood samples were dispensed into vacuum blood collection tubes and centrifuged (4°C, 3000 rpm, 10 min) immediately. The serum samples were separated and stored at -30°C until use. 25(OH)D, urea nitrogen, creatinine, triglyceride, total cholesterol, albumin, and high-density lipoprotein cholesterol levels were measured by SRL Inc. (Tokyo, Japan). The serum 25(OH)D levels were measured by chemiluminescent enzyme immunoassay (CLEIA) using the measuring device Lumipulse G1200 (Fujirebio, Tokyo, Japan).

#### BDHQ

Habitual diet during the preceding month was assessed using a BDHQ (9). The BDHQ included questions about the frequency of consumption of 58 foods and beverages. The 58 foods were classified into 15 food groups (i.e., (1) cereals (2) potatoes, and starches, (3) sugars and sweeteners, (4) pulses, (5) green and yellow vegetables, (6) other vegetables, (7) fruits, (8) fish, mollusks, and crustaceans, (9) meat, (10) egg, (11) milk and milk products, (12) fats and oils, (13) confectionery, (14) beverages, (15) seasoning and spices) mainly according to the definition of food groups by Kobayashi *et al.* (9), and the weight of intake for each food group was calculated. Nutritional intake was calculated using a commercial computer algorithm for the BDHQ, which was based primarily on the Standard Tables of Food Composition in Japan (10).

#### Questionnaire survey on eating habits

A questionnaire that was developed based on the 2008–2015 National Health and Nutrition Survey in Japan was used to assess the eating habits of the study participants. The questions included the following: (Q1) Do you usually eat breakfast? (almost every day, skip 2–3 days a week, skip 4–5 days a week, rarely), (Q2) Do you usually snack between meals (including midnight snacks)? (at least twice daily, more than once but less than twice daily, more than twice but less than seven times a week, less than twice a week), (Q3) Do you usually skip meals? (at least one meal daily, at least four but less than seven meals a week, at least two but less than four meals a week, less than twice a week), (Q4) Do you usually start dinner after 9:00 p.m.? (almost every day, 4–5 times a week, 2–3 times a week, rarely), (Q5) How many days a week do you eat a combination of a staple meal, a main dish, and a side dish at least twice a day? (almost every day, 4–5 times a week, 2–3 times a week, rarely).

#### Body measurements

After measuring the study participants' height and weight, body fat mass, body fat percentage, muscle mass, and lean body mass were measured using InBody 270 (InBody Japan Inc.). Body mass index (BMI) and lean and muscle percentage were calculated using the following formulas: BMI (kg/m<sup>2</sup>) = weight (kg)/height (m)<sup>2</sup>, lean percentage (%) = lean mass (kg)/weight (kg) × 100, and muscle percentage (%) = muscle mass (kg)/weight (kg) × 100.

#### Statistical analysis

The baseline and dietary characteristics of the study participants are presented as the mean ± standard deviation (SD). The correlation between the serum 25(OH)D levels and nutrient intake, intake of each food group, and body composition

was calculated using single regression analysis. To analyze these associations in more detail, the study participants were divided into tertiles based on the serum 25(OH)D levels of the target population (Tertile1, <11.9 ng/mL; Tertile2, 12.1–15.7 ng/mL; Tertile3, > 15.9 ng/mL). The highest (Tertile3) and lowest (Tertile1) serum 25(OH)D groups were compared for nutrient intake, intake of each food group, body composition, and eating habits, because the serum 25(OH)D levels were biased toward low values and the difference between the lowest (Tertile1) and moderate (Tertile2) groups was small. For each subgroup of the serum 25(OH)D tertiles, the data for all continuous variables were tested for normal distribution via the Shapiro–Wilk test. The differences in variables that were normally distributed in both subgroups were assessed using independent t-tests, while the variables that were not normally distributed in one or both subgroups were assessed using Wilcoxon rank-sum tests. The association between eating habits and the serum 25(OH)D levels was determined using Pearson's chi-square test. All statistical analyses were performed using JMP pro version 16.0.0 (SAS Institute Japan Ltd.), and statistical significance was set at a P value of <0.05.

## RESULTS

#### Characteristics of the study participants

Table 1 shows the characteristics of the study participants. The mean ± SD of age and BMI of the 83 participants were 21.4 ± 0.7 years and 20.3 ± 2.5 kg/m<sup>2</sup>, respectively. Moreover, 77% of the participants had appropriate weights (18.5 ≤ BMI < 25.0), 18% were underweight (BMI < 18.5), and 4.8% were obese (25 ≤ BMI). The mean ± SD of serum-25(OH)D levels was 14.1 ± 4.3 ng/mL; 88% of the study participants did not reach 20 ng/mL, the borderline between deficient and insufficient, and none reach the 30 ng/mL threshold for sufficient (Figure1-A). None of the

Table 1. Characteristics of study subjects

		All subjects (n = 83)	
Age	(year)	21.4	± 0.7
Anthropometric			
Body height	(cm)	159.5	± 5.2
Body weight	(kg)	51.7	± 7.0
BMI	(kg/m <sup>2</sup> )	20.3	± 2.5
Body fat mass	(kg)	13.9	± 4.7
Body fat percentage	(%)	26.5	± 5.7
Lean mass	(kg)	37.8	± 3.8
Lean percentage	(%)	73.5	± 5.6
Muscle mass	(kg)	35.6	± 3.6
Muscle percentage	(%)	69.2	± 5.4
Blood			
25(OH)D	(ng/ml)	14.1	± 4.3
Triglyceride	(mg/dl)	65	± 25.1
Total-Cholesterol	(mg/dl)	177.3	± 23.4
HDL-Cholesterol	(mg/dl)	67.9	± 10.6
Albumin	(g/dl)	4.7	± 0.2
Blood urea nitrogen	(mg/dl)	11.5	± 2.9
Creatinine	(mg/dl)	0.63	± 0.09

All values expressed as mean ± SD.

BMI, body mass index; 25(OH)D, 25-hydroxyvitamin D; HDL-Cholesterol, high-density lipoprotein cholesterol.

study participants deviated from the reference values for the other items.

#### Habitual dietary vitamin D intake

Table 2 shows the characteristics of the habitual dietary intake obtained from the BDHQ.

The mean  $\pm$  SD vitamin D intake was  $10.0 \pm 6.7 \mu\text{g}$ , while 50.6% of the study participants met the desired adequate intake (AI) based on the Dietary Reference Intakes for Japanese (2020) of  $8.5 \mu\text{g}$  for vitamin D (Figure 1-B). The mean  $\pm$  SD intake of fish, mollusks, and crustaceans, main sources of vitamin D<sub>3</sub>, and other vegetables, including mushrooms, main sources of vitamin

D<sub>2</sub>, were  $56.7 \pm 35.4 \text{ g}$  and  $141.7 \pm 79.1 \text{ g}$ , respectively.

#### Association of serum 25(OH)D levels and habitual dietary intake

To identify the dietary factors associated with the serum 25(OH)D levels, the serum 25(OH)D levels and habitual nutritional intake and intake of each food groups were applied to a single regression analysis. No significant association was observed between the serum 25(OH)D levels and vitamin D intake (simple regression analysis,  $r = -0.007$ ,  $p = 0.9532$  [Table 3]), and no significant correlations were observed in the intake of other nutrients or food groups. However, a negative trend was observed between the serum 25(OH)D levels and the intake of

**Table 2.** Characteristics of nutrients and food groups intakes of study subjects

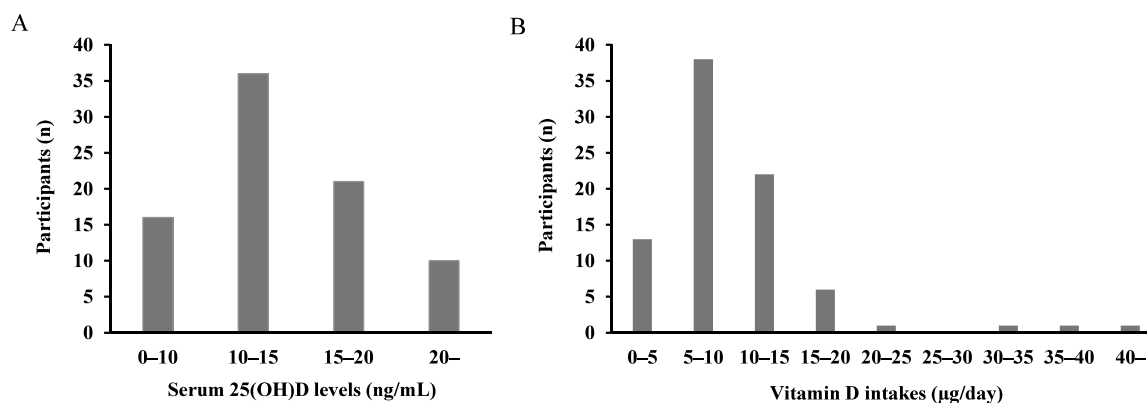
		All subjects (n=83)	
Nutrients			
Energy	(kcal)	1628.4	$\pm$ 437.2
Protein	(g)	61.5	$\pm$ 19.7
Fat	(g)	54.5	$\pm$ 16.6
Carbohydrate	(g)	212.0	$\pm$ 63.6
Vitamin D	( $\mu\text{g}$ )	10.0	$\pm$ 6.7
Food groups			
Cereals	(g)	358.7	$\pm$ 138.0
Potatoes and starches	(g)	46.5	$\pm$ 36.3
Sugars and sweeteners	(g)	3.3	$\pm$ 2.9
Pulses	(g)	47.9	$\pm$ 34.1
Green and yellow vegetables	(g)	114.9	$\pm$ 76.2
Other vegetables	(g)	141.7	$\pm$ 79.1
Fruits	(g)	69.9	$\pm$ 56.4
Fish, mollusks and crustaceans	(g)	56.7	$\pm$ 35.4
Meat	(g)	77.7	$\pm$ 37.3
Egg	(g)	47.7	$\pm$ 23.7
Milk and milk products	(g)	121.3	$\pm$ 77.6
Fats and oils	(g)	11.8	$\pm$ 5.1
Confectionery	(g)	39.7	$\pm$ 25.0
Beverages	(g)	428.3	$\pm$ 280.3
Seasoning and spices	(g)	189.2	$\pm$ 127.5

All values expressed as mean  $\pm$  SD.

**Table 3.** Correlation coefficients between serum 25(OH)D and habitual dietary intakes

		25(OH)D	
		r	p value
Nutrients			
Energy	(kcal)	-0.062	0.5748
Protein	(g)	-0.056	0.6127
Fat	(g)	-0.035	0.7542
Carbohydrate	(g)	-0.066	0.5544
Vitamin D intake	( $\mu\text{g}$ )	-0.007	0.9532
Food groups			
Cereals	(g)	-0.089	0.4245
Potatoes and starches	(g)	-0.009	0.9375
Sugars and sweeteners	(g)	-0.044	0.6912
Pulses	(g)	-0.124	0.2625
Deeply colored vegetables	(g)	-0.096	0.3892
Other vegetables	(g)	0.110	0.3238
Fruits	(g)	-0.062	0.5767
Fish, mollusks and crustaceans	(g)	-0.023	0.8331
Meat	(g)	-0.065	0.5570
Egg	(g)	0.051	0.6466
Milk and milk products	(g)	0.058	0.6051
Fats and oils	(g)	-0.201	0.0685
Confectionaries	(g)	0.089	0.4217
Beverages	(g)	-0.214	0.0513
Seasoning and spices	(g)	-0.052	0.6425

25(OH)D, 25-hydroxyvitamin D.



**Figure 1.** Distribution of the serum 25(OH)D levels and vitamin D intake of the study participants. A : serum 25(OH)D levels. B : vitamin D intake. The scale on the horizontal axis means higher than or equal to the lower number and less than the higher number.

*fats and oils* (simple regression analysis,  $r = -0.201$ ,  $p = 0.0685$  [Table3]) and *beverages* (simple regression analysis,  $r = -0.214$ ,  $p = 0.0513$  [Table 3]). For further analysis, we compared the habitual nutritional intake and intake of each food groups in the lowest (Tertile1) and highest (Tertile3) serum 25(OH)D tertile groups via independent t-tests or Wilcoxon rank-sum test. The group with lower serum 25(OH)D levels tended to consume more of *beverages* than the group with higher serum 25(OH)D levels (Wilcoxon rank-sum test,  $p = 0.0868$  [Table 4]), but no significant differences were observed in the intake of other nutrients or food groups.

*Association of serum 25(OH)D levels and eating habits*

The association between the results of the dietary habits questionnaire and serum 25(OH)D levels was analyzed using Pearson’s chi-square test. Based on the results, no differences were observed between the two groups (Tertile1 and Tertile3) in terms of breakfast intake habits, frequency of snacking, skipping meals, late dinners and balanced meal intake (Table 5).

*Association of serum 25(OH)D levels and body compositions*

In the analysis of serum 25(OH)D levels and body compositions, body fat mass, body fat percentage were significantly negatively associated with the serum 25(OH)D levels : simple regression analysis, body fat mass ( $r = -0.216$ ,  $p < 0.05$ ), body fat percentage ( $r = -0.301$ ,  $p < 0.01$ ), (Table 6). Conversely, positive and significant correlations with serum 25(OH)D levels were observed for lean percentage and muscle percentage (simple regression analysis, lean percentage [ $r = 0.292$ ,  $p < 0.01$ ], muscle percentage [ $r = 0.301$ ,  $p < 0.01$ ]) (Table 6). Moreover, in the comparison by tertile groups, the highest 25(OH)D level group (Tertile3) showed a statistically lower body fat mass, body fat percentage, and BMI and statistically higher lean percentage and muscle percentage than the lowest 25(OH)D level group did (Tertile1) (independent t-tests, body fat percentage, lean percentage, and muscle percentage [ $p < 0.01$ ]; Wilcoxon rank-sum test, BMI [ $p < 0.05$ ] and body fat mass [ $p < 0.01$ ] (Table 7).

**Table 4.** Habitual dietary intakes of study sbjects and comparison with serum 25(OH)D levels

25(OH)D levels	(ng/ml)	All sbjects (n=83)	Tertile 1 (n=27)	Tertile 2 (n=29)	Tertile 3 (n=27)	p value
<b>Nutrients</b>						
Energy	(kcal)	1628.4 ± 437.2	1551.6 ± 333.3	1780.8 ± 562.6	1541.6 ± 333.4	0.9125
Protein	(g)	61.5 ± 19.7	59.6 ± 16.2	64.8 ± 24.9	59.7 ± 16.5	0.9311
Fat	(g)	54.5 ± 16.6	53.0 ± 16.9	55.9 ± 18.6	54.4 ± 14.5	0.5334
Carbohydrate	(g)	212.0 ± 63.6	199.1 ± 45.9	242.9 ± 77.8	191.8 ± 49.8	0.5738
Vitamin D intake	(µg)	10.0 ± 6.7	9.0 ± 6.5	11.0 ± 8.3	10.1 ± 4.7	0.1003
<b>Food groups</b>						
Cereals	(g)	358.7 ± 138.0	340.0 ± 116.5	426.1 ± 157.2	305.2 ± 107.0	0.2576
Potatoes and starches	(g)	46.5 ± 36.3	42.3 ± 31.2	52.5 ± 39.5	44.3 ± 37.8	0.7743
Sugars and sweeteners	(g)	3.3 ± 2.9	3.2 ± 2.2	4.0 ± 4.1	2.8 ± 1.7	0.6038
Pulses	(g)	47.9 ± 34.1	53.2 ± 39.1	46.2 ± 36.1	44.4 ± 26.3	0.9242
Deeply colored vegetables	(g)	114.9 ± 76.2	120.4 ± 90.8	108.1 ± 72.6	116.7 ± 65.7	0.6780
Other vegetables	(g)	141.7 ± 79.1	130.5 ± 74.4	142.7 ± 84.5	151.9 ± 79.0	0.3502
Fruits	(g)	69.9 ± 56.4	73.2 ± 63.8	73.7 ± 56.7	62.7 ± 49.4	0.8220
Fish, mollusks and crustaceans	(g)	56.7 ± 35.4	49.3 ± 32.3	64.6 ± 43.3	55.4 ± 27.6	0.1414
Meat	(g)	77.7 ± 37.3	79.4 ± 48.6	77.2 ± 28.3	76.4 ± 33.9	0.6842
Egg	(g)	47.7 ± 23.7	47.7 ± 20.0	44.6 ± 20.9	50.9 ± 29.7	0.9236
Milk and milk products	(g)	121.3 ± 77.6	107.2 ± 62.8	124.7 ± 93.4	131.7 ± 72.8	0.1909
Fats and oils	(g)	11.8 ± 5.1	12.4 ± 6.4	12.1 ± 4.4	11.0 ± 4.4	0.7952
Confectionalies	(g)	39.7 ± 25.0	36.2 ± 22.4	42.1 ± 28.4	40.7 ± 24.1	0.6339
Beverages	(g)	428.3 ± 280.3	461.7 ± 273.1	487.1 ± 352.2	331.8 ± 158.1	0.0868
Seasoning and spices	(g)	189.2 ± 127.5	178.5 ± 126.1	234.7 ± 126.1	150.9 ± 119.5	0.2682

The p-values indicate the results of comparing Tertile 1 and Tertile 3.

Table 5. Eating habits of study subjects and comparison with serum 25(OH)D levels

		Almost everyday	Skip 2-3 times / week	Skip 4-5 times / week	Rarely	Total	p value	
Breakfast intake	Tertile 1	n	20	5	0	27	0.5937	
		%	37.0	9.3	0.0	3.7		50
	Tertile 3	n	23	3	0	1		27
		%	42.6	5.6	0.0	1.9		50
		twice or more times / day	Once / day	2-6 times / week	0-1 times / week	Total	p value	
Snacking	Tertile 1	n	3	16	6	2	27	0.2628
		%	5.6	29.6	11.1	3.7	50	
	Tertile 3	n	7	13	7	0	27	
		%	13.0	24.1	13.0	0.0	50	
		Once or more times / day	4-6 times / week	2-3 times / week	0-1 times / week	Total	p value	
With no meal	Tertile 1	n	2	0	4	21	27	0.2762
		%	3.7	0.0	7.4	38.9	50	
	Tertile 3	n	0	1	2	24	27	
		%	0.0	1.9	3.7	44.4	50	
		Almost everyday	4-5 times / week	2-3 times / week	Rarely	Total	p value	
Dinner intake after 9 pm	Tertile 1	n	2	5	10	10	27	0.9135
		%	3.7	9.3	18.5	18.5	50	
	Tertile 3	n	1	6	9	11	27	
		%	1.9	11.1	16.7	20.4	50	
		Almost everyday	4-5 times / week	2-3 times / week	Rarely	Total	p value	
Valanced meal intake	Tertile 1	n	10	5	7	5	27	0.8007
		%	18.5	9.3	13.0	9.3	50	
	Tertile 3	n	13	3	7	4	27	
		%	24.1	5.6	13.0	7.4	50	

Table 6. Correlation coefficients between serum 25(OH)D and body compositions

		25(OH)D	
		r	p value
BMI	(kg/m <sup>2</sup> )	-0.215	0.0508
Body fat mass	(kg)	-0.216	0.0328 *
Body fat percentage	(%)	-0.301	0.0056 **
Lean mass	(kg)	0.074	0.5039
Lean percentage	(%)	0.292	0.0073 **
Muscle mass	(kg)	0.083	0.4548
Muscle percentage	(%)	0.301	0.0057 **

\* p&lt;0.05, \*\* p&lt;0.01.

25(OH)D, 25-hydroxyvitamin D ; BMI, body mass index.

Table 7. Body compositions of study subjects and comparison with serum 25(OH)D levels

		All subjects (n=83)	Tertile 1 (n=27)	Tertile 2 (n=29)	Tertile 3 (n=27)	p value
25(OH)D levels	(ng/ml)	6.4 - 24.7	6.4 - 11.9	12.1-15.7	15.9 - 24.7	
BMI	(kg/m <sup>2</sup> )	20.3 ± 2.5	21.3 ± 2.9	20.0 ± 2.1	19.7 ± 2.2	0.0213 *
Body fat mass	(kg)	13.9 ± 4.7	15.7 ± 5.3	13.5 ± 3.8	12.6 ± 4.4	0.0053 **
Body fat percentage	(%)	26.5 ± 5.7	28.7 ± 5.9	26.4 ± 5.1	24.3 ± 5.3	0.0047 **
Lean mass	(kg)	37.8 ± 3.8	38.1 ± 3.9	37.0 ± 3.3	38.3 ± 4.2	0.8252
Lean percentage	(%)	73.5 ± 5.6	71.3 ± 5.9	73.6 ± 5.1	75.6 ± 5.3	0.0058 **
Muscle mass	(kg)	35.6 ± 3.6	35.8 ± 3.7	34.8 ± 3.1	36.1 ± 4.0	0.7792
Muscle percentage	(%)	69.2 ± 5.4	67.0 ± 5.6	69.3 ± 4.8	71.3 ± 5.1	0.0049 **

\* p&lt;0.05, \*\* p&lt;0.01. BMI, body mass index.

The p-values indicate the results of comparing Tertile 1 and Tertile 3.

## DISCUSSION

In this study, we investigated the serum 25(OH)D levels of young Japanese women and the factors associated with these levels. Because this study was conducted in July, it is inferred that the serum 25(OH)D levels of the study participants were relatively high during the year considering seasonal variations. However, about 88% of the study participants in this study were deficient (<20 ng/mL) in vitamin D and about 12% had insufficient ( $\geq 20$  – <30 ng/mL) levels; none of the participants showed sufficient ( $\geq 30$  ng/mL) levels. This is generally consistent with the findings of a report by Ota *et al.* suggesting that 99.3% of young Japanese women are insufficient/deficient in vitamin D (11), suggesting that vitamin D deficiency/insufficiency is widespread among young Japanese women.

However, approximately 50% of the study participants met the desired AI (8.5  $\mu$ g) of vitamin D based on the Dietary Reference Intakes for Japanese (2020). It is estimated that the AI based on the Dietary Reference Intakes for Japanese (2020) is insufficient to maintain a serum 25(OH)D level of 20 ng/mL or higher. Although differences in certain factors, such as the amount of sunlight, the nature of the skin, and dietary habits between Americans and Japanese, were must be taken into account, a U.S. study of young women receiving 10–60  $\mu$ g of vitamin D in a stepwise fashion suggested that 10  $\mu$ g of vitamin D in Caucasian woman and 20–40  $\mu$ g in African–American women are needed to maintain serum 25(OH)D levels equal to or above 20 ng/mL (12). Moreover, a study conducted in Japan targeting men and women aged 21–76 years old also revealed that administering 25  $\mu$ g of vitamin D for 6 months increased the serum 25(OH)D levels from 12.1 ng/mL to 28.5 ng/mL (13). Therefore, these findings suggest that it may be difficult to maintain serum 25(OH)D levels above 20 ng/mL with a vitamin D intake of 8.5  $\mu$ g in Japanese.

In addition, no significant association was found between the serum 25(OH)D levels and nutritional intake or intake of food groups. It has been shown that the primary factor determining the vitamin D nutritional status is exposure to sunlight (14). Conversely, studies that investigated the effect of vitamin D intake and UV exposure on serum vitamin D levels have suggested that not only UV but also the oral intake of vitamin D is also associated with the serum 25(OH)D levels (15). Because our study was conducted in July, it is possible that the serum 25(OH)D levels of the study participants were strongly influenced by sunlight exposure and were not associated with the dietary factors.

However, several concerns exist in making such a prediction. First, the assessment of the vitamin D intake of the study participants was inaccurate. Vitamin D is characterized by a large day-to-day variation with approximately 80% of its intake derived from seafood (16). Therefore, it is difficult to determine the habitual intake of vitamin D, and a validity study of the BDHQ also reported more than 50% of overestimating vitamin D intake compared with the estimated vitamin D intake in the food weighed method (17). Moreover, since we cannot rule out the possibility of underreporting, which frequently occurs in the dietary survey, it is possible that vitamin D intake is not accurately assessed due to these multiple factors. Second, since we could not assess the opportunity for sun exposure in this study, it is not known how much endogenous vitamin D was produced. Moreover, it has been shown that vitamin D production may be inhibited by UV irradiation to the skin through the use of sunscreens (18). Since our study was conducted in young women, it is also possible that the serum 25(OH)D levels of the study participants were strongly influenced by their use of sunscreen. Currently, a questionnaire has been developed in Japan to easily

identify the risk of vitamin D deficiency. This questionnaire includes questions about the frequency of intake of vitamin D-rich foods and exposure to sunlight (19). In future, vitamin D-specific questionnaires should be used to identify factors related to the nutritional status of vitamin D.

Furthermore, we analyzed the association between the serum 25(OH)D levels and eating habits, considering that the infrequent breakfast intake or frequency of late dinners may reflect the morningness or eveningness of the study participants. Whether are the morningness or eveningness may be related to the UV exposure status of the study participants. However, no association was observed between the frequency of breakfast intake, snacking, skipping meals, late dinners, or balanced meals and the serum 25(OH)D levels. Since these results may be due to biased responses and the small number of the study participants, further research with a larger study cohort is needed.

The serum 25(OH)D levels were not associated with dietary factors but were positively associated with muscle and lean body percentage, and negative associations with body fat mass and body fat percentage were observed. These results suggested that the higher the serum 25(OH)D levels, the higher the percentage of muscle and lean tissue of the study participants. Previously, it has been shown that low serum 25(OH)D levels increase the risk of decreased grip strength and decreased limb skeletal muscle mass in the elderly (20). Another Japanese study on elderly participants also reported significant differences in the time to stand on one leg with open eyes, walking speed, and the number of falls between the groups with serum 25(OH)D levels above and below 20 ng/mL (21). Furthermore, an inverse association was observed between serum 25(OH)D levels and the incidence of low muscle mass in a cohort study of adults in Korea (22). Since skeletal muscle mass peaks in the 20s and then declines significantly with age (23), it is important to maintain sufficient muscle mass from a healthy young age to prevent sarcopenia and locomotive syndrome in the future. Although the role of vitamin D on bone and skeletal muscle health in the elderly has gained research interest, our results revealed that maintaining proper vitamin D nutritional status not only in the elderly but also in young people contributes to the maintenance of the skeletal muscle system and may lower the risk of sarcopenia and locomotive syndrome.

In conclusion, none of the study participants showed sufficient serum 25(OH)D levels, suggesting that vitamin D deficiency/insufficiency is widespread among young Japanese women. The serum 25(OH)D levels were not associated with dietary factors but were positively associated with muscle and lean body percentage; however, they were negatively associated with body fat mass and body fat percentage. These results suggested that the higher the serum 25(OH)D levels, the higher the percentage of muscle and lean tissue of the study participants. Our results indicated that maintaining proper vitamin D nutritional status not only in the elderly but also in young people contributes to the maintenance of the skeletal muscle system and may lower the risk of sarcopenia and locomotive syndrome.

## CONFLICT OF INTEREST

All authors declare no conflict of interest.

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