Demographic and Lifestyle Factors Associated with Vitamin D Status in Pregnant Japanese Women

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Summary Maternal vitamin D deficiency causes pregnancy complications and delayed skeletal development in offspring. This study aimed at identifying demographic and lifestyle factors associated with vitamin D status in pregnant Japanese women. A total of 284 healthy pregnant women in the second trimester were recruited at a university hospital in Tokyo, between June 2010 and July 2011. Serum 25-hydroxyvitamin D (25(OH)D) concentrations were measured using chemiluminescent immunoassay. We assessed vitamin D intake using a self-administered diet history questionnaire and asked participants about lifestyle variables, including daily duration of sunlight exposure and supplement use. The mean (SD) serum 25(OH)D concentration was 9.8 (4.7) ng/mL. Almost 60% of the participants had severe vitamin D deficiency (measured as 25(OH)D<10 ng/mL). Multiple regression analysis showed that multigravidity, pre-pregnancy non-underweight status, higher energy-adjusted vitamin D intake, and use of vitamin D supplements were correlated with higher serum 25(OH)D concentrations (β=0.245, β=-0.119, β=0.226, and β=0.197, respectively). In the summer investigation, women with longer durations of sunlight exposure had significantly higher serum 25(OH)D concentrations (β=0.201) that were unrelated to the factors outlined previously. In the winter investigation, women with a high education level had higher serum 25(OH)D concentrations than others (β=0.330). Our results would be useful for identifying pregnant women at a high risk of low vitamin D status, such as primigravidae and those with pre-pregnancy underweight status, low education level, low vitamin D intake, and short durations of sunlight exposure.

Key Words dietary intake, pregnancy, sunlight exposure, supplement, vitamin D

Vitamin D has drawn attention as a significant nutrient to prevent pregnancy complications and health problems in offspring (1, 2). For instance, maternal vitamin D deficiency has been speculated to cause endothelial dysfunction, a key feature of preeclampsia, through a proinflammatory response and increase in oxidative stress (3). Its deficiency also leads to low birth size through the inhibition of bone mineral accrual of the fetus (4).

Maintaining maternal circulating 25-hydroxyvitamin D (25(OH)D) concentrations above 30.0 ng/mL is recommended (5). However, the high ratio of severe vitamin D deficiency (25(OH)D<10.0 ng/mL) in pregnant women is a significant concern in many countries (6–8). In Japan, two studies showed that the mean serum 25(OH)D concentrations during pregnancy were only 9.5–14.5 ng/mL (9, 10). The concentrations were especially lower in winter. To improve the vitamin D status of pregnant Japanese women, identification of populations at a high risk of vitamin D deficiency and implementation of appropriate intervention are necessary. In general, vitamin D status depends on vitamin D intake (vitamin D2 and D3) and vitamin D production in the skin through exposure to sunlight (11). Although vitamin D status was reported to be affected by age, body mass index (BMI), physical activity, and season (12–15), factors associated with vitamin D status vary with the target population and latitude. Among pregnant Australian women, low serum 25(OH)D concentrations were significantly predicted by non-Caucasian, high BMI, non-use of vitamin D supplements, and winter season (14). Among non-pregnant Japanese women, young age, low dietary vitamin D intake, low walk counts, and long sedentary activity time correlated with low serum 25(OH)D concentrations (16, 17). The walk counts and sedentary activity time were presumed to reflect the amount of exposure to sunlight. However, it remains unclear which factors are associated with vitamin D status in pregnant Japanese women, the extent to which those factors are associated with vitamin D status, and whether the factors vary with the season.

The objective of the present study was to examine demographic and lifestyle factors associated with...
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serum 25(OH)D concentrations among pregnant Japanese women in consideration of the season. The results would be useful for the identification of populations at high risk of vitamin D deficiency.

MATERIALS AND METHODS

Study participants. The present study was conducted at a university hospital in Tokyo (35.4° N), Japan, between June 2010 and July 2011. We recruited all healthy Japanese women with singleton pregnancies at 19–23 wk gestation, who had an antenatal medical checkup and had a plan to give birth at the research hospital. The exclusion criteria included diabetes, hypertension, psychological diseases, age less than 20 y, and poor Japanese reading ability. Each participant was given detailed information about the study protocol before she gave written informed consent. The study procedures and protocol were approved by the ethics committee of the Graduate School of Medicine at the University of Tokyo. This study was conducted in accordance with the Helsinki Declaration of the World Medical Association.

Participants answered questionnaires while waiting for their antenatal medical checkup. Women who did not have sufficient time to complete the questionnaires filled them out after returning home and then submitted them by mail. To reduce subject burden, non-fasting blood samples were drawn during a routine antenatal blood test.

Preparation and analysis of blood biomarkers. Within 8 h of blood collection, blood samples were centrifuged for 10 min at 3,000 rpm to separate the serum and then stored at −80°C until measurement. The serum 25(OH)D concentrations were measured using chemiluminescent immunoassay (LIAISON 25 OH Vitamin D TOTAL Assay; Diasorin, Salugia, Italy). The assay was conducted by Kyowa Medex Co., Ltd., Tokyo, Japan. The 25(OH)D concentration is the best marker for evaluating circulating vitamin D insufficiency (18). This assay monitors both vitamin D₂ and D₃. The intraassay and interassay coefficients of variation of 25(OH)D concentrations were less than 8%. The linear dynamic range was 4.0–150.0 ng/mL. The values measured using the LIAISON assay were reported to be 13.5% lower than those measured using a liquid chromatography-tandem mass spectrometry method, which has been as widely used as the LIAISON assay (19). However, strong correlations between the values measured by these methods have been established (19).

Diet history questionnaire. The diet history questionnaire (DHQ) is a 22-page structured questionnaire designed to assess dietary intake in the past month for the Japanese adult population (20–22), and its validity for assessing vitamin D intake among pregnant Japanese women has been established (10). The DHQ includes questions about eating frequency, food portion size, general dietary behavior, and primary cooking methods. The food items and portion sizes were derived from primary data of the National Nutrition Survey of Japan and various recipe books for Japanese dishes (20). Estimate for energy and vitamin D intakes were based on the Japanese standard food composition tables (23). Eight responses for eating frequency are listed, ranging from “more than twice per day” to “almost never.” Five responses for portion size are listed, ranging from “less than half of a general portion size” to “more than 1.5 times a general portion size.” We used the density method for energy adjustment of vitamin D intake to reduce the impact of misreporting (24). The nutrient density was calculated as the ratio of vitamin D content to total energy (μg/1,000 kcal). The total dietary intake and energy-adjusted intake did not include vitamin D intake from supplements.

We excluded participants who reported an extremely unrealistic energy intake from the analyses: that is, the reported energy intake was less than half the energy requirement for the lowest physical activity category or more than 1.5 times the energy requirement for moderate physical activity category according to the Dietary Reference Intakes (DRIs) for Japanese (25, 26).

Pregnancy physical activity questionnaire. The level of physical activity was measured using a modified Japanese version of the Pregnancy Physical Activity Questionnaire (PPAQ-J) (27, 28), which is the only validated instrument designed specifically to assess the physical activity of pregnant Japanese women (29). The PPAQ-J is a semiquantitative questionnaire to determine the time spent participating in 33 activities including household/caring giving, occupation, sports/exercise, transportation, and inactivity. For each activity, participants were asked to select a category for the amount of time spent in that activity per day or week during the preceding 1-mo period. Total physical activity was calculated as a grand total of the average metabolic equivalent hours per day (METs·h/d) for each physical activity.

General questionnaires. Information on the demographic and lifestyle variables such as age, gestational age, education level, and smoking status was collected by a self-administered questionnaire. We calculated the pre-pregnancy BMI from self-reported pre-pregnancy weight and height. The BMI was classified according to World Health Organization criteria: underweight (BMI< 18.5 kg/m²), normal weight (18.5≤BMI<25.0 kg/m²), and overweight or obese (BMI≥25.0 kg/m²).

We also asked about the presence of pregnancy-associated nausea, use of vitamin D supplements, duration of sunlight exposure, and use of skin protection from sunburn (i.e., use of sunscreen or a parasol) during the preceding 1-mo period. We asked the participants who used vitamin D supplements to provide their supplements’ brand names. Duration of sunlight exposure was assessed separately for weekdays and weekends, and we calculated the mean daily sunlight exposure duration as (minutes of sunlight exposure on weekdays×5) + (minutes of sunlight exposure on weekends×2)/7. For frequency of the use of skin protection from sunburn, participants could choose from the following categories: not used at all, sometimes used, or always used. The latter 2 categories were classified as the use of skin protection from sunburn.
Table 1. Characteristics of participants.

<table>
<thead>
<tr>
<th></th>
<th>All participants (n=284)</th>
<th>Summer investigation (n=157)</th>
<th>Winter investigation (n=127)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD or n (%)</td>
<td>Min.</td>
<td>Max.</td>
</tr>
<tr>
<td>Age [y]</td>
<td>34.8±4.1</td>
<td>23</td>
<td>46</td>
</tr>
<tr>
<td>Gestational age [wk]</td>
<td>20.3±1.1</td>
<td>19</td>
<td>23</td>
</tr>
<tr>
<td>Parity: Multigravida</td>
<td>n (%)</td>
<td>103 (37.3)</td>
<td>62 (39.5)</td>
</tr>
<tr>
<td>Currently married</td>
<td>n (%)</td>
<td>283 (99.6)</td>
<td>156 (99.4)</td>
</tr>
<tr>
<td>Education [n (%)]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (High school)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Middle (Junior or technical college)</td>
<td>120 (42.3)</td>
<td>62 (39.5)</td>
<td>58 (45.7)</td>
</tr>
<tr>
<td>High (College or university)</td>
<td>140 (49.3)</td>
<td>83 (52.9)</td>
<td>57 (44.9)</td>
</tr>
<tr>
<td>Annual household income</td>
<td>n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low (&lt;5 million Japanese yen)</td>
<td>48 (16.9)</td>
<td>29 (18.5)</td>
<td>19 (15.0)</td>
</tr>
<tr>
<td>High (≥5 million Japanese yen)</td>
<td>236 (83.1)</td>
<td>128 (81.5)</td>
<td>108 (85.0)</td>
</tr>
<tr>
<td>Height [cm]</td>
<td>159±5</td>
<td>147</td>
<td>175</td>
</tr>
<tr>
<td>Pre-pregnancy body mass index (BMI) [kg/m²]</td>
<td>20.4±2.4</td>
<td>15.8</td>
<td>29.1</td>
</tr>
<tr>
<td>Underweight (BMI&lt;18.5 kg/m²)</td>
<td>n (%)</td>
<td>56 (19.7)</td>
<td>30 (19.1)</td>
</tr>
<tr>
<td>Normal BMI (18.5 kg/m² ≤ BMI&lt;25.0 kg/m²)</td>
<td>n (%)</td>
<td>210 (73.9)</td>
<td>118 (75.2)</td>
</tr>
<tr>
<td>Overweight (BMI≥25.0 kg/m²)</td>
<td>n (%)</td>
<td>18 (6.3)</td>
<td>9 (5.7)</td>
</tr>
<tr>
<td>Serum 25-hydroxyvitamin D [ng/mL]</td>
<td>9.8±4.7</td>
<td>4.0</td>
<td>27.2</td>
</tr>
<tr>
<td>Energy intake¹ [kcal/d]</td>
<td>1,811±405</td>
<td>1,009</td>
<td>3,016</td>
</tr>
<tr>
<td>Daily vitamin D intake</td>
<td>µg/d</td>
<td>6.3±3.1</td>
<td>0.9</td>
</tr>
<tr>
<td>Energy-adjusted vitamin D intake</td>
<td>µg/1,000 kcal</td>
<td>3.5±1.5</td>
<td>0.6</td>
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<tr>
<td>Smoking during pregnancy</td>
<td>n (%)</td>
<td>3 (1.1)</td>
<td>2 (1.3)</td>
</tr>
<tr>
<td>Use of vitamin D supplements</td>
<td>n (%)</td>
<td>41 (14.4)</td>
<td>25 (15.9)</td>
</tr>
<tr>
<td>Having pregnancy-associated nausea</td>
<td>n (%)</td>
<td>86 (30.3)</td>
<td>47 (29.9)</td>
</tr>
<tr>
<td>Working</td>
<td>n (%)</td>
<td>139 (48.9)</td>
<td>70 (44.6)</td>
</tr>
<tr>
<td>Duration of sunlight exposure² [min/d]</td>
<td>11±8.5</td>
<td>5</td>
<td>523</td>
</tr>
<tr>
<td>Use of skin protection from sunburn³</td>
<td>n (%)</td>
<td>243 (85.6)</td>
<td>142 (90.4)</td>
</tr>
<tr>
<td>Total physical activity⁴ [METs·h/d]</td>
<td>26±9.1</td>
<td>9.3</td>
<td>62.4</td>
</tr>
<tr>
<td>Season of investigation: Summer (from April to September)</td>
<td>n (%)</td>
<td>157 (55.3)</td>
<td></td>
</tr>
</tbody>
</table>

¹ Dietary intakes were assessed using a self-administered diet history questionnaire.
² Duration of sunlight exposure was assessed separately for weekdays and weekends. We calculated the mean daily sunlight exposure duration as (minutes of sunlight exposure on weekdays×5 + minutes of sunlight exposure on weekends×2)/7.
³ Use of skin protection from sunburn was assessed as use of sunscreen or parasol.
⁴ Total physical activity level was assessed using the pregnancy physical activity questionnaire.
We divided the investigation into 2 categories based on cumulative ultraviolet (UV)-B dose in each season: the summer investigation (from April to September) and the winter investigation (from October to March). The monthly cumulative UV-B dose in 2011 was 18.5–28.1 kJ/m² from April to September and 4.9–13.0 kJ/m² from October to March at Tsukuba, which is near Tokyo (30).

Statistical analyses. A χ² test was used to examine the association between 2 categorical variables. Spearman’s rank correlation coefficients were calculated to investigate whether there were associations between demographic and lifestyle factors and serum 25(OH)D concentrations. In addition, we performed multiple regression analyses to identify the related factors of serum 25(OH)D concentrations. Variables with $p<0.20$ for the Spearman’s rank correlation coefficient were entered as independent variables into the multiple regression models. Categorical independent variables were considered as dummy variables. For a non-normal distribution of a continuous variable, multiple regression analyses were performed after log transformation of the variable to achieve a normal distribution. If the correlation coefficient of any 2 independent variables was $>0.70$ in the Spearman’s rank correlation coefficient, either variable was excluded from the multiple regression model in consideration of multicollinearity.

In addition, logistic regression analysis was performed to identify the factors related to severe vitamin D deficiency (measured as 25(OH)D<10.0 ng/mL). Variables with $p<0.20$ for binary logistic regression analyses were entered as independent variables into the multiple logistic regression models. Adjusted odds ratio (AOR) and 95% confidence interval (95% CI) are shown.

Analyses were conducted using Statistical Package for Social Sciences for Windows, version 15.0 (SPSS Japan Inc., Tokyo, Japan). All statistical tests were two-tailed. We considered $p$ values $<0.05$ to be statistically significant.

RESULTS

A total of 321 pregnant women met the inclusion criteria. Of these women, 299 (93.1%) gave their written informed consent. Fifteen women were excluded from the analysis. 14 for missing data and 1 for reporting an unrealistically low energy intake. Ultimately, data from 284 healthy pregnant women (88.5%) were analyzed.

The characteristics of the participants are shown in Table 1. The mean (SD) age was 34.8 (4.1) y, and there were 103 multigravidae (37.3%). Eighty-six pregnant women (30.3%) reported having pregnancy-associated nausea in the preceding 1-mo period. Almost 1 in 5 women (19.7%) was underweight before pregnancy. The mean (SD) serum 25(OH)D concentration and energy-
adjusted vitamin D intake were 9.8 (4.7) ng/mL and 3.5 (1.5) μg/1,000 kcal, respectively. The serum 25(OH)D concentrations of 271 participants (95.4%; summer: 146, winter: 125) were below 20.0 ng/mL, and those of 166 participants (58.5%; summer: 89, winter: 77) were below 10.0 ng/mL. In addition, 203 women (71.5%; summer: 89, winter: 77) were below 20.0 ng/mL, and those of 271 participants (95.4%; summer: 146, winter: 125) were below 25.0 ng/mL. The mean serum 25(OH)D concentrations among women with low, middle, and high durations of sunlight exposure were significantly higher among multigravidae than primigravidae (31.0 vs. 22.9 METs·h/d and 136.7 vs. 96.6 min/d, respectively). No other variables were associated with parity.

Table 2 shows the Spearman’s rank correlation coefficients between demographic and lifestyle variables and serum 25(OH)D concentrations. Multigravidae and users of vitamin D supplements had significantly higher serum 25(OH)D concentrations than others (r_s=0.296 and r_s=0.158, respectively). In addition, energy-adjusted vitamin D intake, duration of sunlight exposure, and total physical activity levels correlated positively with serum 25(OH)D concentrations (r_s=0.203, r_s=0.216, and r_s=0.238, respectively). A positive correlation was also found between daily duration of sunlight exposure and daily total activity levels (r_s=0.291, data not shown). In the summer investigation, higher pre-pregnancy BMI was associated with higher serum 25(OH)D concentrations. The mean serum concentrations and energy-adjusted intake of vitamin D among women who were underweight, normal weight, and overweight before pregnancy were 8.6, 10.6, and 12.7 ng/mL and 3.1, 3.5, and 3.4 μg/1,000 kcal, respectively. In the winter investigation, women with higher education levels had higher serum 25(OH)D concentrations (r_s=0.187). The mean serum 25(OH)D concentrations among women with low, middle, and high education levels were 6.9, 8.9, and 9.9 ng/mL, respectively (p=0.080). In addition, the skin protection usage...
rates were 33.3%, 84.5%, and 84.2% in the women with low, middle, and high education levels, respectively (p<0.001). Other variables were not associated with education levels.

In the multiple regression analysis, multigravidae and women with higher energy-adjusted vitamin D intake and use of vitamin D supplements had higher serum 25(OH)D concentration (β=0.245, β=0.226, and β=0.197, respectively, Table 3). Women with pre-pregnancy underweight status had significantly lower vitamin D concentrations (β=−0.119). Among the participants in the summer investigation, multigravidity, pre-pregnancy overweight status, higher energy-adjusted vitamin D intake, and longer duration of sunlight exposure were correlated with higher serum 25(OH)D concentrations (β=0.243, β=0.143, β=0.172, and β=0.201, respectively). In the winter investigation, multigravidity, a high education level, higher energy-adjusted vitamin D intake, and use of vitamin D supplements were correlated with higher serum 25(OH)D concentrations (β=0.192, β=0.330, β=0.260, and β=0.298, respectively).

Participants with severe vitamin D deficiency (25(OH)D <10.0 ng/mL) had significantly lower daily vitamin D intake (5.9 vs. 6.9 μg/d, p=0.003), energy-adjusted vitamin D intake (3.3 vs. 3.7 μg/1000 kcal, p=0.010), total physical activity level (24.1 vs. 28.5 METs-h/d, p=0.001), and duration of sunlight exposure (97.2 vs. 130.9 min/d, p=0.001) than the other participants. Multigravidae and users of vitamin D supplements were less likely to have severe vitamin D deficiency than others (42.7% vs. 67.4% and 36.6% vs. 62.1%, respectively). The rate of severe vitamin D deficiency was not affected by the season in which the investigation was undertaken. In a multiple logistic regression analysis, women who were multigravidae (AOR=0.432, 95%CI=0.240–0.777, p=0.005), had a higher energy-adjusted vitamin D intake (AOR=0.829, 95%CI=0.693–0.992, p=0.040), used vitamin D supplements (AOR=0.258, 95%CI=0.124–0.538, p=0.001), had longer durations of sunlight exposure (AOR=0.997, 95%CI=0.993–1.000, p=0.048), and had higher total physical activity levels (AOR=0.967, 95%CI=0.936–0.998, p=0.039) were less likely to have severe vitamin D deficiency during pregnancy.

DISCUSSION

We could identify the factors associated with circulating vitamin D status among pregnant Japanese women. It is notable that almost 60% of participants had severe vitamin D deficiency (25(OH)D<10.0 ng/mL) and more than 95% of participants had 25(OH)D<20.0 ng/mL, even after considering the 13.5% lower values resulting from our 25(OH)D measurement method compared with those obtained using other methods. Appropriate nutritional counseling is absolutely imperative during pregnancy, especially for pregnant women at high risk of vitamin D deficiency.

Multigravidae and women with higher dietary vitamin D intake had higher serum 25(OH)D concentrations, a finding that agrees with previous studies (13, 32). In the present study, a correlation between parity and 25(OH)D concentrations was found even after adjusting for total physical activity level and duration of sunlight exposure, which differed significantly depending on parity. These results imply that other factors specific to multigravidae might affect vitamin D status. Although these factors were unclear in our study, healthcare providers should pay attention to primigravidae as a potentially high risk population for poor vitamin D status. The observed relationship between dietary vitamin D intake and serum 25(OH)D concentration was valid in consideration of the sources of vitamin D. Previous studies have reported this relationship in countries with high fish consumption and in non-pregnant Japanese women (13, 16, 33–35). The contribution ratio of dietary vitamin D intake to circulating 25(OH)D concentrations varies substantially by country because the consumption of fish and seafood, which are major sources of vitamin D, differs throughout the world. According to a study of pregnant women in Australia, vitamin D intake had no effect on serum concentration (14), perhaps because vitamin D production in the skin is the major source of vitamin D compared with the relatively low vitamin D intake in Australia (36). On the other hand, results of our study showed a significant correlation between dietary intake and serum concentrations, which could be attributed to the relatively high fish consumption and therefore, high vitamin D intake in Japan (37). However, more than 70% of our participants had a lower vitamin D intake than the AI of 7.0 μg/d (25). In addition, the mean vitamin D intake in the women with severe vitamin D deficiency was lower than that in the women without severe vitamin D deficiency. Healthcare providers need to recommend that pregnant women consciously consume vitamin D-rich foods such as fish, seafood, eggs, and mushrooms to prevent vitamin D deficiency.

Women with pre-pregnancy underweight status had lower 25(OH)D concentrations. In addition, the women with pre-pregnancy overweight status tended to have higher 25(OH)D concentrations in the summer investigation. These results were different from those of previous studies, which reported that obesity increases the risk of circulating vitamin D insufficiency because of vitamin D storage in adipose tissue and accordingly bioavailability reduction (13, 14, 38, 39). The following reasons might be responsible for these differences in the results observed. First, there were no obese participants (BMI > 30.0 kg/m²) in our study. Therefore, a drastic reduction in bioavailability may not occur. Second, BMI does not necessarily reflect the amount of adipose tissue. Third, an increase in BMI and adipose mass during pregnancy, which depends on an individual’s gestational weight gain and nutritional intake, was not taken into consideration (40, 41). Fourth, women with a pre-pregnancy underweight status are likely to have low dietary intakes or low vitamin D synthesis because of their lifestyle, although we did not observe such relationships to be significant. Thus, further investigation
of the association between BMI and vitamin D status, considering the adipose tissue accretion accompanying pregnancy, is required.

In the summer investigation, women with longer duration of sunlight exposure had higher serum 25(OH)D concentrations. The correlation between duration of sunlight exposure and vitamin D status was verified only in summer, despite the same mean duration of sunlight exposure in both seasons, possibly because vitamin D production in the skin is likely to be higher in summer than in winter due to the larger amount of UV radiation per unit time of sunlight exposure in summer. This higher vitamin D production in summer could contribute greatly to higher 25(OH)D concentrations. Exposure to sunlight, resulting in moderate vitamin D production in the skin, is an easy and effective method to satisfy the requirements for vitamin D (42). To obtain sufficient vitamin D in Japan, the Ministry of the Environment recommends, a brief exposure to sunlight equivalent to placing both hands in the sun for 15 min or in the shade for 30 min in a day, in addition to adequate dietary vitamin D intake (43). On the other hand, a great number of Japanese women use skin protection to avoid sunburn for either cosmetic reasons or to prevent skin cancer and skin damage. Skin protection, however, inhibits vitamin D production (44). A negative attitude about sunburn and the subsequent use of skin protection might reduce the contribution of sunlight exposure to vitamin D status. In fact, the mean 25(OH)D concentrations between the summer and winter investigations did not differ greatly, as opposed to the difference in cumulative UV-B dose by season. Healthcare providers should explain to pregnant women the positive health benefits of sunlight exposure and adequate amounts and effective ways to acquire sunlight exposure that is healthy for both mothers and fetuses.

In the winter investigation, a high education level was associated with high serum 25(OH)D concentrations, similar to that reported by previous studies (45, 46). Education levels did not correlate with dietary intake, use of supplements, or duration of sunlight exposure. On the other hand, participants with a low education level had a significantly lower skin protection usage rate than women with middle or high education levels. Generally, a lower skin protection usage rate leads to a higher vitamin D concentration for the same sunlight exposure duration. However, the participants with low education levels had lower vitamin D status. Although we could not determine the reason for this contradictory result, we should consider this as a high-risk population with poor vitamin D status. In addition, use of vitamin D supplements was correlated with serum 25(OH)D concentrations in multiple regression analysis. We presume that the seasonal differences in the ratio of vitamin D supplements contributing to circulating vitamin D concentrations would indicate that ingested vitamin D was more effective than vitamin D synthesized endogenously when sunlight exposure is low. On the other hand, independent of season, women who used vitamin D supplements were less likely to have severe vitamin D deficiency. Taking vitamin D supplements of 3.4–5.0 μg/d, in addition to dietary intake, would be sufficient to prevent vitamin D deficiency. However, the daily vitamin D amount from supplements alone is not adequate during pregnancy; therefore, a higher dietary vitamin D intake and sufficient sunlight exposure, in addition to taking supplements, are important for ensuring higher vitamin D status.

Total physical activity significantly correlated with severe vitamin D deficiency, which has been shown in previous studies of pregnant women in other countries (47, 48). This correlation is observed because physical activity levels reflect the amount of exposure to sunlight in some degree. We used the questionnaire to garner information on sunlight exposure since directly measuring actual sunlight exposure in free-living is impossible. Thus, such an indirect variable linked to sunlight exposure could be observed as a significant variable.

This study had two limitations. First, the characteristics of the participants might be biased because the research hospital was a university hospital in an urban area. Actually, the mean age of participants was a little older than that of national reports (34.5 y vs. 31.2 y) (49). Second, measurement values using the LIAISON 25 OH Vitamin D TOTAL Assay might be lower than those obtained using other vitamin D measurement methods (19).

**CONCLUSIONS**

The serum 25(OH)D concentrations significantly correlated with parity, pre-pregnancy BMI, education levels, dietary vitamin D intake, use of vitamin D supplements, duration of sunlight exposure, and total physical activity in pregnant Japanese women. These results would be useful for the identification of populations at high risk of vitamin D deficiency during pregnancy. Most of the participants had severe vitamin D deficiency, regardless of season. This showed that even sunlight exposure in summer does not effectively provide sufficient vitamin D when using skin protection and receiving a vitamin D intake lower than the AI. Healthcare providers need to inform pregnant women of the necessity of consciously increasing vitamin D intake and ensuring adequate sunlight exposure to prevent vitamin D deficiency.

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**Competing interests**

The authors declare that they have no competing interests.

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