Low Serum 25-Hydroxyvitamin D Concentrations Are Associated with Increased Likelihood of Having Depressive Symptoms among Japanese Workers\textsuperscript{1,2}

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Abstract

Background: Accumulating evidence suggests a protective role of vitamin D against mood disorders; however, epidemiologic studies are scarce in working populations.

Objective: We investigated cross-sectionally the association of serum vitamin D status and depressive symptoms among Japanese workers.

Methods: Participants were 1786 employees (9\% women), aged 19–69 y, who received health check-ups and participated in a nutrition and health survey. Serum 25-hydroxyvitamin D [25(OH)D] concentrations were measured with the use of a competitive protein binding assay. Depressive symptoms were assessed by using the Center for Epidemiologic Studies Depression (CES-D) scale. Logistic regression was used to estimate ORs with adjustment for potential confounding variables including dietary factors.

Results: Overall, 92\% of study participants had suboptimal vitamin D status [25(OH)D < 30 \(\mu\)g/L]. Depressive symptoms were inversely associated with 25(OH)D. Compared with those with a 25(OH)D concentration of < 20 \(\mu\)g/L, multivariable-adjusted ORs (95\% CIs) for depressive symptoms (CES-D scale score $\geq 16$) were 0.75 (0.59, 0.95) and 0.66 (0.41, 1.06) for those with a 25(OH)D concentration of 20–29 \(\mu\)g/L and $\geq 30$ \(\mu\)g/L, respectively ($P$-trend = 0.01). After further adjustment for leisure-time physical activity and shift work (factors closely related to photo-initiated vitamin D production), the OR (95\% CI) for the highest category of 25(OH)D was 0.70 (0.43, 1.14). The association between 25(OH)D and depressive symptoms appears to be linear, according to restricted cubic spline regression.

Conclusion: Results suggest that lower concentrations of circulating vitamin D are associated with increased likelihood of having depressive symptoms among apparently healthy workers.


Keywords: cross-sectional studies, depressive symptoms, Japanese, worker, 25-hydroxyvitamin D

Introduction

Depression is a common mental disorder. The global prevalence of major depressive disorders in 2010 was 4.4\% and was shown to peak between 20 and 64 y of age (1). Depressive disorders were the second leading cause of years lived with disability in 2010, and the largest proportion of years lived with disability from these disorders occurred among working adults (2). Thus, depression has been linked to substantial losses in work performance (3). An identification of modifiable risk factors for these disorders is thus a priority issue in the occupational health setting.

Several lines of evidence from mechanistic, experimental, and epidemiologic studies point to the important role of vitamin D in the development of depression. Vitamin D receptors and vitamin D activating enzyme 1α-hydroxylase are present in the human brain (4), and vitamin D receptor knockout mice were shown to exhibit depressive behaviors (5). A meta-analysis of observational studies (6) reported a statistically significant increase in the prevalence or risk of depressive symptoms or depression in persons with low circulating concentrations of 25-hydroxyvitamin D [25(OH)D], which is a measure of the body’s vitamin D status.

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\textsuperscript{6} Abbreviations used: CES-D, Center for Epidemiologic Studies Depression; MET, metabolic equivalent; 25(OH)D, 25-hydroxyvitamin D.
a marker of systemic vitamin D exposure, and such an association has been replicated in more recent studies (7–13).

Several important issues remain unsettled, however. First, epidemiologic studies to date have been performed mainly among community members or the elderly, but evidence is scarce among employees of a manufacturing company in the nonferrous metal industry and their affiliated companies in the Kanto region of Japan. This study was conducted at the time of the worksite health examination, which employees were obliged to receive on a yearly basis. Major occupational exposures (% workers exposed in the major company) were organic solvents (15%), heavy lifting (10%), lasers (10%), noise (5%), and ionizing radiation (4%). We invited all employees undergoing the health checkup to participate in the survey and asked them to fill out 2 types of study-specific questionnaires: one for diet and another for health-related lifestyle in general. Of 2828 checkup attendants (11% of those who were invited), 2728 (96%) agreed to participate in the survey. We asked participants to donate 7-mL venous blood for study. We also obtained health examination data containing anthropometric and biochemical data and information on medical history. The study protocol was approved by the Ethics Committee of the National Center for Global Health and Medicine of Japan. Written informed consent was obtained from each participant.

We excluded 100 participants with a history of the following diseases diagnosed by a physician: cancer (n = 20), cardiovascular disease (n = 25), chronic hepatitis (n = 2), chronic pancreatitis (n = 3), kidney disease (n = 11), or psychiatric disorders including depression and anxiety disorders (n = 45); some participants had ≥2 of these conditions. Of the remaining 2062 participants, we sequentially excluded participants who lacked information on blood vitamin D (n = 215), depression status (n = 3), and covariates used in the analysis (n = 58), leaving 1786 subjects (1622 men (91%) and 164 women (9%)), aged 19–69 y, for analysis.

**Vitamin D measurement.** Venous blood donated for the study was drawn into a vacuum tube and centrifuged to separate serum. After the measurement of insulin, the remaining serum sample was stored at −80°C until analysis. 25(OH)D concentrations were determined at an external laboratory (LSI Medience Corporation, Tokyo, Japan) with the use of a competitive protein binding assay, with the intra-assay coefficients of variation of 10.9% at 13.3 μg/L and 8.9% at 21.3 μg/L.

**Assessment of depressive symptoms.** Depressive symptoms were assessed with the use of a Japanese version (22) of the Center for Epidemiologic Studies Depression (CES-D) scale (23). In a validation study for the Japanese version, a CES-D scale score cut-off of ≥16 showed a sensitivity of 88.2% and specificity of 84.8% (22). This scale consists of 20 items covering 6 typical symptoms of depression: depressed mood, feelings of guilt or worthlessness, feelings of helplessness or hopelessness, psychomotor retardation, loss of appetite, and sleep disturbance experienced during the preceding week. We calculated the total CES-D scale score for each participant according to standard procedure. Participants with a CES-D scale score of ≥16 were regarded as having depressive symptoms.

**Other variables.** Body height and weight were measured to the nearest 0.1 cm and 0.1 kg, respectively, while the participants wore light clothes and no shoes. BMI was calculated as the body weight in kilograms divided by the square of body height in meters. Marital status, job grade, shift work, overtime work, smoking status, alcohol consumption, sleep duration, work-related (work, domestic housework, and commuting to and from work) and leisure-time physical activities, and psychological work environment were assessed via a lifestyle questionnaire. Work-related and leisure-time physical activities were each expressed as the sum of metabolic equivalents (METs) multiplied by the duration of time engaged across activities with different intensity. Psychological work environment was assessed by using the Job Content Questionnaire (24), and the job strain score was calculated according to a standard procedure. Dietary habits during the preceding 1-mo period were assessed via a validated brief self-administered diet history questionnaire, and dietary intakes for 58 food and beverage items, energy, and selected nutrients were estimated with the use of an ad hoc computer algorithm (25).

**Statistical analyses.** The characteristics of the study population in either proportion or mean were presented according to 25(OH)D category. Although there is no consensus on optimal concentrations of circulating vitamin D, 25(OH)D concentrations of <20, 20–29, and ≥30 μg/L are considered by most experts to be deficient, insufficient, and sufficient, respectively (26). Logistic regression was used to calculate OR and its 95% CI for depressive symptoms according to 25(OH)D. We adjusted for age (y, continuous), sex, and factory in the basic model (model 1). In model 2, we additionally adjusted for marital status (married or other), job grade (high, middle, or low), overtime work (<10, 10–29, or ≥30 h/mo), job strain (quartile), smoking (never smoked, quit smoking, current smoker consuming <20 cigarettes/d, or current smoker consuming ≥20 cigarettes/d), alcohol drinking [nondrinkers; occasional drinkers (1–3 times/mo); or regular drinkers consuming 23, 24–45, or ≥46 g ethanol/d], sleep duration (<6, 6–6.9, or ≥7 h/d), work-related physical activity (METs h/d, quartile), BMI (kg/m²), continuous), energy intake (kcal/d), and daily dietary intakes of folate (μg/1000 kcal), vitamin B-6 (mg/1000 kcal), n–3 PUFAs (% energy), magnesium (mg/1000 kcal), and iron (mg/1000 kcal). Higher concentrations of these micronutrients in dietary intake or blood have been shown to be associated with lower depressive symptoms (17, 18, 27–29). In model 3, leisure-time physical activity (METs h/wk, quartile) and shift work (yes or no), both of which are closely related to the chance of sunlight exposure and thus can directly influence 25(OH)D concentration, were...
Additionally adjusted for. Trend association was assessed by assigning the median 25(OH)D concentration to each exposure category and modeling this variable as continuous. We repeated the above analyses according to quartile of 25(OH)D. In a sensitivity analysis, we included only nonusers of multivitamin or vitamin B supplements (n = 1617). We also performed restricted cubic spline regression to evaluate the shape of the relation between 25(OH)D and the odds of depressive symptoms, by assigning 14.9 μg/L to the reference value of 25(OH)D and 14.9, 20.9, and 28.7 μg/L (10th, 50th, and 90th percentiles, respectively) to the 3 knots. Two-sided P values < 0.05 were regarded as statistically significant. Analyses were performed with the use of Statistical Analysis System version 9.3 (SAS Institute) and Stata version 13.1 (StataCorp).

Results

The proportion of participants with vitamin D deficiency (<20 μg/L) and insufficiency (20–29 μg/L) were 41.6% and 50.8%, respectively. As shown in Table 1, compared with participants with high 25(OH)D concentrations, those with low 25(OH)D concentrations tended to be young, female, and smokers, were more likely to be engaged in shift work and overtime work, and slept less. They were less likely to be married and drink alcohol and engaged in leisure-time physical activity. In regard to diet, participants with low 25(OH)D concentrations had lower dietary intakes of energy and vitamin D, and they tended to consume lower amounts of vitamin B-6, n–3 PUFAs, magnesium, and iron.

As shown in Table 2, analyses for the predefined cut-off for 25(OH)D showed that serum 25(OH)D concentrations were significantly and inversely associated with the likelihood of having depressive symptoms when adjusted for sex, age, and workplace (model 1, P-trend < 0.001). In model 2, which additionally included marital status, job grade, overtime work, job strain, smoking, alcohol drinking, sleep duration, work-related physical activity, BMI, and dietary factors, ORs (95% CIs) of depressive symptoms for the lowest (<20 μg/L) through highest (≥30 μg/L) category of 25(OH)D were 1 (reference), 0.75 (0.59, 0.95), and 0.66 (0.41, 1.06), respectively (P-trend = 0.01).

After further adjustment for shift work and leisure-time physical activity (model 3), the OR (95% CI) for the highest concentration of 25(OH)D was 0.70 (0.43, 1.14), whereas the trend association remained significant (P-trend = 0.04). In a sensitivity analysis of nonusers of multivitamin or vitamin B supplements (n = 1617), the association was slightly stronger; the ORs (95% CIs) of depressive symptoms for the lowest through highest category of 25(OH)D in model 2 were 1 (reference), 0.72 (0.56, 0.93), and 0.57 (0.33, 0.96), respectively (P-trend = 0.004).

A statistically significant, albeit less clear, inverse association was observed in an analysis using the quartile of 25(OH)D instead of the predefined category. In model 2 (without adjustment for shift work and leisure-time physical activity), the ORs (95% CIs) of depressive symptoms for the lowest through highest quartile were 1 (reference), 1.10 (0.82, 1.50), 0.85 (0.62, 1.17), and 0.74 (0.53, 1.14), respectively (P-trend = 0.03). In this model that included 25(OH)D as a continuous variable instead of a categorical one, the multivariable-adjusted OR decreased by 0.21 (95% CI: 0.01, 0.37) for each 10-μg/L increment of 25(OH)D.

According to the result of cubic spline regression analysis (Figure 1), the association between serum 25(OH)D concentration and depressive symptoms appeared to be linear; the odds of depressive symptoms steadily decreased with increasing 25(OH)D concentrations.

Discussion

In a cross-sectional study of Japanese employees, 92% had suboptimal vitamin D status [25(OH)D <30 μg/L]. There was a significant inverse trend association between circulating vitamin D

## Table 1

| Characteristics of study participants according to serum 25(OH)D concentrations
<table>
<thead>
<tr>
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<tbody>
<tr>
<td>Serum 25(OH)D, μg/L</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>Age, y</td>
</tr>
<tr>
<td>Sex (men)</td>
</tr>
<tr>
<td>Factory A (survey in April 2012)</td>
</tr>
<tr>
<td>Married</td>
</tr>
<tr>
<td>Low job grade</td>
</tr>
<tr>
<td>Shift work (yes)</td>
</tr>
<tr>
<td>Overtime work (≥30 h/mo)</td>
</tr>
<tr>
<td>Job strain</td>
</tr>
<tr>
<td>Current smoker</td>
</tr>
<tr>
<td>Alcohol consumption (≥1 d/wk)</td>
</tr>
<tr>
<td>Sleep duration [&lt;6 h/d]</td>
</tr>
<tr>
<td>Leisure-time physical activity, METs h/wk</td>
</tr>
<tr>
<td>Work-related physical activity, METs h/d</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
</tr>
<tr>
<td>Dietary intake (per day)</td>
</tr>
<tr>
<td>Total energy, kcal</td>
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<tr>
<td>Folate, μg/1000 kcal</td>
</tr>
<tr>
<td>Vitamin B-6, mg/1000 kcal</td>
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<tr>
<td>n–3 PUFAs, % energy</td>
</tr>
<tr>
<td>Magnesium, mg/1000 kcal</td>
</tr>
<tr>
<td>Iron, mg/1000 kcal</td>
</tr>
<tr>
<td>Vitamin D, μg/1000 kcal</td>
</tr>
</tbody>
</table>

*Values are means±SDs or n (%). MET, metabolic equivalent; 25(OH)D, 25-hydroxyvitamin D.*
concentrations and prevalence of depressive symptoms in fully adjusted models, although ORs for the highest concentration of 25(OH)D were not statistically significant. This is among few studies of workers that addressed the association between circulating vitamin D and depression.

The present finding agrees with those of a meta-analysis (6) and recent prospective (10) and cross-sectional studies (7–13). Many of these previous investigations were performed in a community setting or among older individuals who are more likely to have comorbidity that may influence both mental function and vitamin D metabolism, leading to a higher likelihood of confounding. We previously reported a higher, albeit statistically not significant, odds of having depressive symptoms among local government employees with lower 25(OH)D concentrations in blood samples collected in summer and late autumn (14). In the present larger-scale study of young and middle-aged workers, 92.4% had either vitamin D deficiency (<20 μg/L), job strain (quartile), smoking (never smoked, quit smoking, current smoker consuming <20 cigarettes/d, or current smoker consuming ≥20 cigarettes/d), alcohol drinking (nondrinker, drinker consuming <23 g ethanol/d, drinker consuming 23–45 g ethanol/d, or drinker consuming ≥46 g ethanol/d), sleep duration (<6, 6–6.9, or ≥7 h/d), work-related physical activity (METs h/d, quartile), BMI (kg/m², continuous), total energy intake, and daily dietary intakes of folate (μg/1000 kcal), vitamin B-6 (mg/1000 kcal), n–3 PUFAs (% energy), magnesium (mg/1000 kcal), and iron (mg/1000 kcal).

<table>
<thead>
<tr>
<th>Predefined category of 25(OH)D, μg/L</th>
<th>&lt;20</th>
<th>20–29</th>
<th>≥30</th>
<th>P-trend1</th>
</tr>
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<tbody>
<tr>
<td>Median</td>
<td>17.0</td>
<td>23.4</td>
<td>32.0</td>
<td>—</td>
</tr>
<tr>
<td>Range</td>
<td>7.9–19.9</td>
<td>20.0–29.9</td>
<td>30.0–38.7</td>
<td>—</td>
</tr>
<tr>
<td>Subjects, n</td>
<td>743</td>
<td>908</td>
<td>135</td>
<td>—</td>
</tr>
<tr>
<td>Cases, n</td>
<td>241</td>
<td>216</td>
<td>28</td>
<td>—</td>
</tr>
<tr>
<td>Model 1: OR (95% CI)2</td>
<td>1.00 [reference]</td>
<td>0.67 (0.54, 0.84)</td>
<td>0.58 (0.38, 0.92)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Model 2: OR (95% CI)3</td>
<td>1.00 [reference]</td>
<td>0.75 (0.59, 0.95)</td>
<td>0.66 (0.41, 1.06)</td>
<td>0.01</td>
</tr>
<tr>
<td>Model 3: OR (95% CI)4</td>
<td>1.00 [reference]</td>
<td>0.78 (0.62, 0.99)</td>
<td>0.70 (0.43, 1.14)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

1 Based on multiple logistic regression analysis with assignment of median value for each category of serum 25(OH)D. 25(OH)D, 25-hydroxyvitamin D.

2 Adjusted for age (y, continuous), sex, and factory.

3 Adjusted for age (y, continuous), sex, factory, marital status (married or other), job grade (high, middle, or low), overtime work (<10, 10–29, or ≥30 h/mo), job strain (quartile), smoking (never smoked, quit smoking, current smoker consuming <20 cigarettes/d, or current smoker consuming ≥20 cigarettes/d), alcohol drinking (nondrinker, drinker consuming <23 g ethanol/d, drinker consuming 23–45 g ethanol/d, or drinker consuming ≥46 g ethanol/d), sleep duration (<6, 6–6.9, or ≥7 h/d), work-related physical activity (METs h/d, quartile), BMI (kg/m², continuous), total energy intake, and daily dietary intakes of folate (μg/1000 kcal), vitamin B-6 (mg/1000 kcal), n–3 PUFAs (% energy), magnesium (mg/1000 kcal), and iron (mg/1000 kcal).

4 Adjusted for variables in model 2, shift work (yes or no), and leisure-time physical activity (METs h/wk, quartile).

This study supports evidence of a link between vitamin D insufficiency and depression in a working population. Various demographic, regional, seasonal, and lifestyle factors are known to be associated with 25(OH)D concentrations (16), and we adjusted for a range of potential confounding variables including dietary factors. In multivariate modeling, however, there is a concern of overadjustment because some factors directly contribute to circulating vitamin D concentrations. In assessing the association between circulating vitamin D and depression, dietary vitamin D intake should not be adjusted for because its effect on mood, if any, is exerted solely by increasing circulating vitamin D. What about factors like leisure-time physical activity and shift work that are closely related to sunlight exposure, which has a greater impact on circulating vitamin D than does dietary vitamin D intake (26)? Shift work and leisure-time physical activity may influence mood via vitamin D–unrelated pathways [i.e., circadian rhythm dysfunction (32) and the activation of endorphin secretion (33)], but they also contribute to mood through their influence on 25(OH)D.
concentrations. Thus, adjustment for these factors would lead to an underestimation of the association between vitamin D and depression, and true estimates may lie between those with adjustment for these factors and those without.

It would be of interest to know whether a widespread definition of vitamin D deficiency [25(OH)D of <20 µg/L] and insufficiency [25(OH)D of 20–29 µg/L (26)], which has been proposed by experts based primarily on findings of bone health, can be applied to mental disorders. In the present study, odds of depressive symptoms decreased in a stepwise manner from the lowest (<20 µg/L) to highest (≥30 µg/L) category of 25(OH)D, giving some support for the use of these cut-offs in relation to mental disorders. In addition, the result of our cubic spline regression (a more suitable technique in assessing the shape of the association) suggests that the association between serum 25(OH)D and depressive symptoms would be linear. Several studies have reported an inverse trend association (7, 8, 13, 34–37), but their data cannot readily be compared to the present study’s data because of the difference in the cut-off of 25(OH)D or the handling of 25(OH)D in the analysis (continuous scale). In a large study of US young adults (38), the odds of depression increased among those with a 25(OH)D concentration of <20 µg/L but not among those with a 25(OH)D concentration of 20–29 µg/L. In another prospective study in the United Kingdom (10), there was an inverse, but nonlinear, association between baseline 25(OH)D and subsequent risk of depression. Because the data on the shape of association are limited and contradictory, more research is clearly needed. Nevertheless, given a high prevalence of vitamin D insufficiency in free-living populations (16, 26), the present finding suggests a large potential for the prevention of depressive disorders through improving vitamin D status.

Major strengths of the present study include a large sample size, a reasonably high participation rate (76%), measurement of blood 25(OH)D (a marker of systemic vitamin D exposure), a relatively homogenous study population (high internal validity), and consideration of a range of potential confounding variables. The short period of survey (April and May) may be an additional advantage because season is an important determinant of both vitamin D concentration and mood and thus may confound the association. We also acknowledge several limitations that warrant mention. First, an association derived from a cross-sectional study does not necessarily imply causality. Workers with depressive symptoms may be more likely to have decreased appetite and may be more reluctant to engage in outdoor activity, leading to vitamin D insufficiency. We confirmed, however, that the inverse association between 25(OH)D and depressive symptoms persisted even after adjustment for energy intake and leisure-time physical activity. Moreover, higher baseline vitamin D concentrations either in diet (39) or blood (10, 40) have been associated with a lower risk of depression in prospective studies, eliminating reverse causation as a probable explanation for the observed association in the present study. Second, depressive symptoms were assessed with the use of the CES-D scale. However, similar associations have been reported in studies that used clinically diagnosed depression as the outcome (12, 35). Third, we measured blood concentrations of vitamin D only at one point in time. Repeated measurements of 25(OH)D could better capture long-term exposure, which may be more relevant to the pathogenesis of depressive symptoms. Fourth, 25(OH)D measurement in this study was not validated with the use of external standards of known vitamin D concentration. Fifth, the possibility of bias because of residual confounding and unmeasured factors cannot be ruled out. Finally, our study was conducted among workers of a manufacturing company; hence, caution should be exercised when generalizing the present finding to the whole working population.

In summary, lower circulating 25(OH)D concentrations were associated with increased likelihood of having depressive symptoms among apparently healthy workers. Prospective studies for clinical depression are needed to confirm the inverse association in the present cross-sectional study.

Acknowledgments

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References


