European Journal of Clinical Nutrition (2013) **67**, 1060–1065

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ORIGINAL ARTICLE

# Serum pyridoxal concentrations and depressive symptoms among Japanese adults: results from a prospective study

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**BACKGROUND/OBJECTIVES:** Vitamin B6 is suggested to have a protective role against depression. However, the association between vitamin B6 intake and depression remains inconclusive, and few studies have examined the relationship between circulating vitamin B6 concentrations and depressive symptoms. Here, we investigated the cross-sectional and prospective associations between serum pyridoxal concentrations and depressive symptoms among Japanese workers.
**SUBJECTS/METHODS:** Participants were 422 municipal employees (aged 21–67 years) who participated in a baseline survey in 2006 for cross-sectional analysis, and 210 subjects without depressive symptoms at baseline (2006) who completed both baseline and follow-up (2009) surveys for prospective analysis. Depressive symptoms were assessed using the Center for Epidemiologic Studies Depression (CES-D) scale. Logistic regression analysis was used to estimate the odds ratio of depressive symptoms (CES-D scale of $\geqslant 19$) according to tertile of serum pyridoxal with adjustment for potential confounding variables.
**RESULTS:** In the cross-sectional analysis, serum pyridoxal concentrations were significantly associated with a decreased prevalence of depressive symptoms ($P$ for trend $= 0.03$); the multivariable-adjusted odds ratio of depressive symptoms for the highest tertile of pyridoxal was 0.54 (95% confidence interval 0.30–0.96) compared with the lowest tertile. In longitudinal analyses, higher serum pyridoxal concentrations at baseline were associated with a trend toward reduced depressive symptoms after 3 years; the multivariable-adjusted odds ratio of depressive symptoms for the highest versus the lowest tertile of pyridoxal concentration was 0.55 (95% confidence interval 0.13–2.32).
**CONCLUSIONS:** A higher vitamin B6 status may be associated with a decreased risk of depressive symptoms in Japanese.

*European Journal of Clinical Nutrition* (2013) **67,** 1060–1065; doi:10.1038/ejcn.2013.115; published online 26 June 2013

**Keywords:** cross-sectional study; depression; Japanese; prospective study; vitamin B6

## INTRODUCTION

Depression is an important public health issue worldwide.[1] Patients with severe depression have limited social life because of the associated symptoms and are at increased risk of suicide.[1] In Japan, the number of inpatients and outpatients with depression is increasing, and the suicide rate is among the highest in the world, with $>30\,000$ deaths from suicide recorded over the last decade.[2] In particular, deaths from suicide have predominantly increased among middle-aged men.[2] Although suicide and depression have shown to be associated with psychosocial factors,[3] epidemiological evidence linking these mental health issues to other modifiable environmental factors, particularly diet, is limited.

B vitamins, including vitamin B6, folate and vitamin B12, are involved in the one-carbon metabolism pathway, which has been suggested to have a role in mental disorders, including depression.[4] Vitamin B6 serves as a cofactor for enzymes involved in the metabolism of homocysteine, which has neurotoxic effects,[5] to cysteine, whereas folate and vitamin B12 are required for the synthesis of methionine from homocysteine.[6] In addition, vitamin B6 is an essential cofactor for tryptophan metabolism and facilitates the conversion of tryptophan to the monoamine neurotransmitter, serotonin.[7] Therefore, vitamin B6 may have a beneficial role in the prevention and treatment of depression. However, a systematic review reported that vitamin B6 had no apparent treatment effect on depression.[8] Although seven cross-sectional[9–15] and two prospective[16,17] studies have examined the association between dietary vitamin B6 intake and depressive symptoms, inconsistent findings were reported. Moreover, only two cross-sectional studies have found an inverse association between depression and plasma concentrations of pyridoxal 5′-phosphate (PLP), which is the major and active form of vitamin B6.[10,18] To date, however, no prospective studies have investigated the relationship between circulating vitamin B6 concentrations and depressive symptoms.

Here, we hypothesized that high serum vitamin B6 is associated with decreased prevalence of depressive symptoms. To test the hypothesis, we investigated the cross-sectional and prospective association of serum pyridoxal concentrations, major vitamin B6 form, with depressive symptoms among Japanese workers. Moreover, we assessed these associations with adjustment for folate and homocysteine, which were associated with depressive symptoms in the same study population.[19,20]

## SUBJECTS AND METHODS

### Study procedure

Health surveys were conducted twice in 2006 and 2009 among employees of two municipal offices in northeastern Kyushu, Japan. Details of the

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Received 7 November 2012; revised 22 May 2013; accepted 23 May 2013; published online 26 June 2013

survey procedure are described elsewhere.[19,20] Briefly, all full-time workers, except those on extended sick leave or maternity leave, were invited to participate in the survey at the time of the periodic health examination (Figure 1). In 2006, of 601 eligible workers, 547 subjects (323 men and 224 women aged 21 to 67 years) participated (91% response rate). In 2009, of 607 eligible workers, 567 subjects (325 men and 242 women aged 20 to 68 years) participated (93% response rate). Participants were asked to complete a survey questionnaire before the health examination and responses were checked by research staff for completeness and, where necessary, clarified by asking the subjects during the examination. We also obtained data that were routinely collected during the health examination, including anthropometric measurements, biochemical data, and information about medical history, smoking and alcohol consumption. In addition, blood and urine samples were obtained. The surveys were conducted at each municipal office. The study protocol was approved by the ethics committee of the National Center for Global Health and Medicine, and written informed consent was obtained from each participant.

## Subjects

The 2006 survey data of 422 participants (245 men and 177 women) after exclusion of 125 participants with missing data for the Center for Epidemiologic Studies Depression (CES-D) scale ($n=3$), serum pyridoxal concentration ($n=119$) and covariates ($n=20$) were used to examine the cross-sectional association between serum pyridoxal and depressive symptoms. A few participants had two or more conditions for exclusion. For the prospective association, we investigated the association between pyridoxal concentrations in 2006 (at baseline survey) and depressive symptoms assessed in 2009 (at follow-up survey). Of 547 subjects who participated in the baseline survey, 461 participants, except those without tenure at follow-up survey ($n=75$) and those on extended sick leave or maternity leave ($n=11$), were invited to complete the follow-up survey (Figure 1). We also excluded 16 participants who did not participate in the follow-up survey, 166 participants with depressive symptoms (CES-D scale of $\geqslant 16$) at baseline, and participants who lacked information on serum pyridoxal ($n=94$) and covariates ($n=12$), leaving a total of 210 participants (125 men and 85 women) for the longitudinal analysis.

## Biochemical measurements

Venous blood (7 ml) was drawn in tube and then transported in a cooler box to the laboratory. The blood was centrifuged for 15 min and the serum was then divided into a maximum of six tubes (0.5 ml each) within 4 h of blood drawing. Five of the tubes were stored at $-30\,^{\circ}C$ (fatty acid composition) or $-80\,^{\circ}C$ (others) until analysis. Serum pyridoxal and homocysteine concentrations were measured by high-performance liquid chromatography, and serum folate concentrations were measured using a chemiluminescent immunoassay at an external laboratory (Mitsubishi Chemical Medience, Tokyo, Japan).

## Depressive symptoms

Depressive symptoms were assessed using the Japanese version[21] of the CES-D scale,[22] which was incorporated into the lifestyle questionnaire in both surveys. The CES-D consists of 20 questions addressing six symptoms of depression, including depressed mood, guilt or worthlessness, helplessness or hopelessness, psychomotor retardation, loss of appetite, and sleep disturbance experienced during the preceding week. Each question is scored on a scale of 0 to 3 according to the frequency of the symptom, with the total CES-D score ranging from 0 to 60. The criterion validity of the CES-D scale has been well established both in Western[22] and Japanese[21] participants. Depressive symptoms were defined as present when subjects had a CES-D score of $\geqslant 16$. A cutoff value of $\geqslant 19$, which may be suitable for Japanese,[23] was also used.

## Dietary assessment

Dietary habits during the preceding month were assessed using a validated brief self-administered diet history questionnaire,[24] which consisted of the following five sections: (1) intake frequency of 46 food and non-alcoholic beverage items; (2) daily intake of rice and miso soup; (3) frequency of alcoholic beverage consumption and amount per drink of five alcoholic beverages; (4) usual cooking methods; and (5) general dietary behavior. Dietary intake for energy and selected nutrients was estimated using a computer algorithm for the brief self-administered diet history questionnaire, with reference to the Standard Tables of Food Composition in Japan.[25] As few subjects used dietary supplements at least once a week, dietary supplements were not incorporated in the nutrient estimation. According to the validation study of the brief self-administered diet history questionnaire using 16-day weighed dietary records as the gold standard, Pearson correlation coefficients for energy-adjusted intake of vitamin B6 and vitamin B12 were 0.48 and 0.40, respectively, in men and 0.49 and 0.31, respectively, in women.[24]

## Other variables

Marital status, job title, job position, and leisure-time and commuting physical activity were asked in the survey questionnaire. Job title was used to create categories for work-related physical activity; sedentary work:

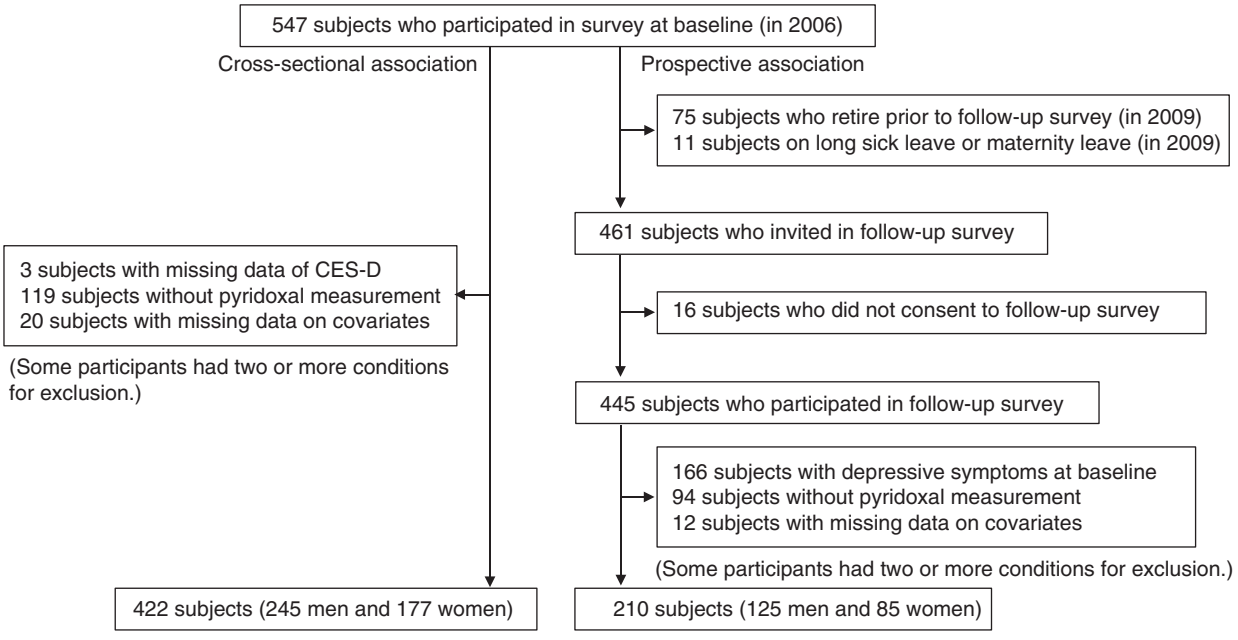


**Figure 1.** Flowchart of study protocol.

managerial and clerical work; physically active work: child-care, school lunch cooking and technical work. For leisure-time and commuting physical activity, participants were asked to provide the number of daily minutes spent walking or cycling during the commute to and from work, and the weekly hours engaged in each of five different leisure activities (walking, low-, moderate- and high-intensity activities, and gardening). The sum of time spent for all commuting and leisure-time activities was expressed in hours per week.

### Statistical analysis

Trend association for participant characteristics according to tertile of serum pyridoxal concentrations was assessed using the Mantel–Haenszel $\chi^2$ test for categorical variables and linear regression analysis for continuous variables. Ordinal numbers 1–3 were assigned to tertile categories of pyridoxal concentrations. To examine the cross-sectional association between pyridoxal concentrations and depressive symptoms in 2006, multiple logistic regression analysis was performed to calculate the odds ratios (OR) and 95% confidence intervals (CI) of depressive symptoms for the tertile of pyridoxal concentrations, using the lowest tertile category as a reference. The first model was adjusted for age (year, continuous), sex and office (A or B), and the second model was further adjusted for marital status (married or unmarried), job position (low or middle and high) work-related physical activity (sedentary or active work), leisure-time and commuting physical activity (0, $>0-<2$ or $\geqslant 2$ h/week), current smoking (yes or no), alcohol consumption (nondrinker, drinker consuming $<20$ g of ethanol/day or drinker consuming $\geqslant 20$ g of ethanol/day), and history of cancer, cardiovascular disease, or mental disease (yes or no), dietary vitamin B12 intake (μg/1000 kcal/day, continuous) and serum folate concentrations (ng/ml, continuous). In the final model, serum homocysteine concentrations (nmol/ml) were added to the second model. We adjusted for folate and homocysteine because they are involved in one-carbon metabolism and have shown to be associated with depressive symptoms.[26,27] Moreover, we repeated the analysis after exclusion of subjects with a high level of C-reactive protein ($>0.5$ mg/l), which is indicative of inflammation, or diabetes to minimize the possibility of reverse causality because these conditions are associated with depression and can also affect serum vitamin B6 concentrations.

For the prospective association analysis, multiple logistic regression analysis was performed to calculate ORs and 95% CIs of depressive symptoms assessed in the follow-up survey for tertile of pyridoxal concentrations at the time of the baseline survey, using the lowest tertile category as a reference. The first model was adjusted for age, sex and office, and the second model was further adjusted for marital status, job position, work-related physical activity, leisure-time and commuting physical activity, current smoking, alcohol drinking, history of cancer, cardiovascular disease, or mental disease, dietary vitamin B12 intake, serum folate concentrations and CES-D score at baseline. In the final model, serum homocysteine concentrations were added. Trends in the cross-sectional and prospective associations were assessed using multiple linear regression analysis, with ordinal numbers 1–3 assigned to tertile categories of pyridoxal concentrations. We repeated the analyses after the exclusion of participants with a history of cancer, cardiovascular disease or mental disease at baseline. We further examined the prospective association between dietary vitamin B6 intake at baseline and depressive symptoms at follow-up among 269 participants for whom data of dietary intake at baseline were available. Two-side *P*-values of $<0.05$ were regarded as statistically significant. All analyses were performed with Statistical Analysis System (SAS) software version 9.1 (SAS Institute, Cary, NC, USA).

## RESULTS

The characteristics of study participants according to tertile categories of serum pyridoxal concentrations are shown in Table 1. Participants with higher pyridoxal concentrations were less likely to be current smokers and were more likely to be physically active and have higher serum folate concentrations than those with lower serum pyridoxal.

Of 422 participants included in the cross-sectional analysis, 159 (37.7%) were identified as having depressive symptoms. The ORs of depressive symptoms according to tertile categories of serum pyridoxal concentrations are shown in Table 2. For the cross-sectional analysis, although the OR of having depressive symptoms among participants with higher pyridoxal concentrations

**Table 1.** Characteristics of study participants according to tertile of serum pyridoxal concentrations ($n=422$)

| | | *Tertile of serum pyridoxal concentrations* | | | |
|---|---|---|---|---|---|
| | *All* | *T1 (low)* | *T2 (mid)* | *T3 (high)* | *Trend P*[a] |
| No of subjects | 422 | 140 | 141 | 141 | |
| Age (year, mean ± s.d.) | 42.9 ± 10.7 | 42.8 ± 10.0 | 42.6 ± 10.6 | 43.4 ± 11.6 | 0.62 |
| Sex (women, %) | 41.9 | 44.3 | 42.6 | 39.0 | 0.37 |
| Office (A, %) | 30.6 | 28.6 | 30.5 | 32.6 | 0.46 |
| Marital status (married, %) | 69.9 | 69.3 | 70.2 | 70.2 | 0.87 |
| Job position (low, %) | 43.8 | 52.1 | 57.4 | 58.9 | 0.26 |
| Smoking status (current, %) | 26.5 | 33.6 | 22.7 | 23.4 | 0.054 |
| Alcohol drinking ($\geqslant 20$ g ethanol/day, %) | 21.3 | 21.4 | 20.6 | 22.0 | 0.91 |
| Work-related physical activity (active work, %) | 22.7 | 25.0 | 18.4 | 24.8 | 0.97 |
| Leisure-time physical activity[b] ($\geqslant 2$ h/week, %) | 34.1 | 27.1 | 36.2 | 39.0 | 0.04 |
| History of diseases[c] (yes, %) | 4.7 | 3.6 | 5.7 | 5.0 | 0.58 |
| Serum folate (ng/ml, mean ± s.d.) | 4.4 ± 2.5 | 3.8 ± 1.7 | 4.4 ± 2.2 | 5.0 ± 3.2 | <0.001 |
| Serum homocysteine (nmol/ml, mean ± s.d.) | 10.9 ± 4.7 | 11.1 ± 5.4 | 11.1 ± 4.2 | 10.4 ± 4.3 | 0.20 |
| *Dietary intake (per day)* | | | | | |
| Total energy (kcal) | 1756 ± 500 | 1742 ± 465 | 1774 ± 511 | 1753 ± 525 | 0.86 |
| Carbohydrate (% of energy) | 52.1 ± 7.9 | 52.8 ± 7.6 | 51.7 ± 8.3 | 51.9 ± 7.6 | 0.33 |
| Fat (% of energy) | 26.9 ± 5.8 | 26.4 ± 5.6 | 27.1 ± 6.2 | 27.3 ± 5.6 | 0.20 |
| Protein (% of energy) | 14.3 ± 2.6 | 14.1 ± 2.7 | 14.3 ± 2.6 | 14.5 ± 2.5 | 0.12 |
| Vitamin B12 (μg/1000 kcal, mean ± s.d.) | 4.8 ± 2.4 | 4.6 ± 2.4 | 4.7 ± 2.2 | 5.1 ± 2.6 | 0.09 |
| Vitamin B6 (mg/1000 kcal, mean ± s.d.) | 0.67 ± 0.16 | 0.65 ± 0.14 | 0.68 ± 0.16 | 0.68 ± 0.17 | 0.09 |
| Vitamin B6 (mg/g protein, mean ± s.d.) | 0.019 ± 0.004 | 0.018 ± 0.003 | 0.019 ± 0.004 | 0.019 ± 0.004 | 0.65 |

Abbreviation: T, tertile. [a]Based on the Mantel–Haenszel $\chi^2$ test for categorical variables and linear regression analysis for continuous variables, with ordinal numbers of 1–3 assigned to the categories of serum pyridoxal concentrations. [b]Leisure-time and commuting physical activity. [c]Diseases included cancer, cardiovascular disease or mental disease.

was approximately 30% lower compared with the lowest tertile category in any model, the trend was not statistically significant (*P* for trend = 0.15 in the fully adjusted model). When a higher cutoff value (CES-D score of ⩾19) was used to define depressive symptoms, the inverse association between serum pyridoxal and depressive symptoms was statistically significant (*P* for trend = 0.03 after adjustment for covariates in model 2). The association did not change after additional adjustment for serum homocysteine (model 3), and similar results were observed after the exclusion of subjects with high C-reactive protein levels or diabetes (data not shown). When subjects with a history of cancer, cardiovascular disease or mental disease ($n = 20$) were excluded, the inverse association did not materially change; the multivariable-adjusted ORs (95% CI) for depressive symptoms (CES-D score of ⩾19) for the lowest through highest tertile categories of serum pyridoxal concentrations were 1.00 (reference), 0.59 (0.34–1.04) and 0.52 (0.29–0.95), respectively (*P* for trend = 0.03).

Of 210 participants without depressive symptoms at baseline, 34 participants (16.2%) were newly identified as having depressive symptoms at the time of the 3-year follow-up survey. As shown in Table 2, no significant association was detected between baseline pyridoxal concentrations and the occurrence of depressive symptoms. However, the OR for depressive symptoms tended to decrease with increasing pyridoxal concentrations at baseline; the multivariable-adjusted ORs (95% CI) for depressive symptoms were 0.60 (0.20–1.80) for CES-D scores of ⩾16 and 0.51 (0.12–2.11) for CES-D scores of ⩾19 in the highest tertile category of serum pyridoxal compared with the lowest category (*P* for trend = 0.38 and 0.34, respectively). After further adjustment for serum homocysteine at baseline, the inverse association was slightly attenuated; the multivariable-adjusted OR for depressive symptoms (CES-D score of ⩾19) of the highest versus lowest tertile was 0.55 (95% CI 0.13–2.32; *P* for trend = 0.40).

When we further examined the association between dietary vitamin B6 intake at baseline and depressive symptoms after 3 years, a suggestive inverse association was observed. The multivariable-adjusted ORs (95% CI) for depressive symptoms (CES-D scores of ⩾16 and ⩾19) of the lowest through highest tertile categories of dietary vitamin B6 intake were 1.00 (reference), 0.93 (0.40–2.16) and 0.37 (0.12–1.10; *P* for trend = 0.09) and 1.00 (reference), 0.99 (0.33–2.99) and 0.44 (0.11–1.86; *P* for trend = 0.30), respectively.

## DISCUSSION

In a cross-sectional analysis of data obtained from a Japanese working population, we observed a significant inverse association between serum pyridoxal concentrations and depressive symptoms (CES-D score of ⩾19) after adjustment for covariates, including folate and homocysteine. Moreover, the occurrence of depressive symptoms after 3 years tended to decrease with higher

**Table 2.** Odds ratios and 95% CI for depressive symptoms according to tertile of serum pyridoxal concentrations: a cross-sectional and prospective association

| | *T1 (low)* | *T2 (mid)* | *T3 (high)* | *Trend* P[a] |
|---|---|---|---|---|
| *Cross-sectional analysis* | | | | |
| Pyridoxal (ng/ml) | 4.7 (2.0–6.3)[b] | 7.5 (6.4–9.8) | 15.2 (>9.8) | |
| No of subjects | 140 | 141 | 141 | |
| *CES-D (15/16)* | | | | |
| No of cases | 63 | 45 | 51 | |
| Model 1[c] | 1.00 (Reference) | 0.58 (0.35–0.94) | 0.70 (0.43–1.13) | 0.14 |
| Model 2[d] | 1.00 (Reference) | 0.53 (0.32–0.89) | 0.68 (0.41–1.15) | 0.14 |
| Model 3[e] | 1.00 (Reference) | 0.53 (0.32–0.88) | 0.69 (0.41–1.15) | 0.15 |
| *CES-D (18/19)* | | | | |
| No of cases | 48 | 32 | 30 | |
| Model 1[c] | 1.00 (Reference) | 0.57 (0.33–0.96) | 0.53 (0.31–0.90) | 0.02 |
| Model 2[d] | 1.00 (Reference) | 0.54 (0.31–0.94) | 0.54 (0.30–0.96) | 0.03 |
| Model 3[e] | 1.00 (Reference) | 0.54 (0.31–0.94) | 0.54 (0.30–0.96) | 0.03 |
| *Longitudinal analysis* | | | | |
| Pyridoxal (ng/ml) | 4.8 (2.2–6.4)[b] | 7.55 (6.5–9.6) | 14.75 (>9.6) | |
| No of subjects | 68 | 72 | 70 | |
| *CES-D (15/16)* | | | | |
| No of cases | 15 | 11 | 8 | |
| Model 1[c] | 1.00 (Reference) | 0.66 (0.27–1.59) | 0.47 (0.18–1.22) | 0.12 |
| Model 2[d] | 1.00 (Reference) | 0.90 (0.33–2.45) | 0.60 (0.20–1.80) | 0.38 |
| Model 3[e] | 1.00 (Reference) | 0.92 (0.34–2.51) | 0.65 (0.22–1.96) | 0.46 |
| *CES-D (18/19)* | | | | |
| No of cases | 9 | 5 | 4 | |
| Model 1[c] | 1.00 (Reference) | 0.49 (0.15–1.57) | 0.41 (0.12–1.42) | 0.14 |
| Model 2[d] | 1.00 (Reference) | 0.69 (0.18–2.59) | 0.51 (0.12–2.11) | 0.34 |
| Model 3[e] | 1.00 (Reference) | 0.70 (0.18–2.65) | 0.55 (0.13–2.32) | 0.40 |

Abbreviations: CES-D, Center for Epidemiologic Studies Depression Scale; CI, confidence interval; T, tertile. [a]Based on multiple logistic regression analysis, assigning ordinal numbers of 1–3 to the tertile categories of serum pyridoxal concentrations. [b]Median (range) of pyridoxal (ng/ml). [c]Adjusted for age (year), sex and office (A, survey conducted in July or B, survey conducted in November). [d]Adjusted for age, sex, office, current smoking (yes or no), alcohol drinking (nondrinker, drinker consuming <20 g of ethanol/day, or drinker consuming ⩾20 g of ethanol/day), work-related physical activity (sedentary or active work), leisure-time and commuting physical activity (0, 0<–<2, or ⩾2 h/week), job position (low or middle and high), marital status (married or unmarried), history of cancer, cardiovascular disease, or mental disease (yes or no), dietary vitamin B12 intake (μg/1000 kcal/day), serum folate concentrations (ng/ml), and CES-D score (continuous) at baseline (for longitudinal analysis only). [e]Additionally adjusted for homocysteine concentrations (nmol/ml).

vitamin B6 status (serum pyridoxal concentrations and dietary intake) at baseline. To our knowledge, this is the first prospective study to examine the association between serum vitamin B6 concentrations and depressive symptoms.

Our finding of a cross-sectional inverse association between serum pyridoxal concentrations and depressive symptoms is consistent with the results of two cross-sectional studies examining the relationship between depression and PLP.[10,18] In a Danish study of 140 men and women aged 19–92 years (median, 75 years), a low plasma PLP concentration was significantly associated with higher depression score assessed by the Major Depression Inventory.[18] A study conducted in the United States also observed that plasma PLP was significantly inversely associated with CES-D score among 869 men and women aged 60 years or over.[10] We further examined the prospective association between depression and serum pyridoxal and observed a decrease (35–60%) in the risk of developing depressive symptoms in participants with higher serum pyridoxal concentrations at baseline, although the analyses did not have sufficient statistical power to detect the reduction in odds with statistical significance. However, the present findings suggest that vitamin B6 may decrease the risk of depression, rather than low vitamin B6 levels resulting from depression.

The present finding of a cross-sectional association between serum pyridoxal and depressive symptoms was statistically significant only when a higher cutoff value (CES-D score of $\geqslant$19) was used to define depressive symptoms. Although a cutoff for CES-D of $\geqslant$16 is widely recommended, a cutoff of $\geqslant$19 has been suggested and validated for use among Japanese workers with a sensitivity and specificity of 92.7% and 91.8%, respectively.[23] We previously reported that serum ferritin concentration was significantly and inversely associated with depressive symptoms assessed using a cutoff value of $\geqslant$19, but not $\geqslant$16, on the CES-D scale.[28] Moreover, an inverse association between serum folate and depressive symptoms became more evident when a cutoff value of $\geqslant$19 was used compared with that of $\geqslant$16.[19,20] Based on the present and previous findings, a CES-D score cutoff value of $\geqslant$19 appears to be more sensitive for detecting associations among the Japanese population.

We previously reported a cross-sectional inverse association between dietary vitamin B6 intake and depressive symptoms in the same study population as the present study.[12] Here, we further found a suggestive inverse association between dietary vitamin B6 intake at baseline and depressive symptoms after 3 years. Of seven cross-sectional studies that have examined the association between vitamin B6 intake and depressive symptoms[9–15], four have observed an inverse association.[10–12,15] Moreover, only two studies have examined the prospective association between vitamin B6 intake and depressive symptoms.[16,17] In a US study, although total vitamin B6 intake (dietary and supplemental) was associated with a decreased risk of depressive symptoms, as assessed by the CES-D, during a mean 7.2-year follow-up period among 3503 men and women aged $\geqslant$65 years, no association for dietary intake of vitamin B6 alone was detected.[17] The authors ascribed the inverse association between total vitamin B6 intake and depressive symptoms to confounding by vitamin B12 contained in multivitamin supplements.[17] In a study among 636 British women aged 53 years, psychological distress, as assessed by the General Health Questionnaire, was not associated with dietary vitamin B6 intake in childhood or adulthood.[16] The inconsistency among studies may be attributable to differences in the length of the follow-up period, age of study subjects, and the assessment of vitamin B6 intake and depressive status.

Meat, fish and pulses are rich in vitamin B6, and the major sources of vitamin B6 in the Japanese diet are vegetables (18.6%), fish and shellfish (16.8%), meat (15.0%), fruit (9.7%) and cereals (8.8%).[29] The association between the intake and blood concentrations of vitamin B6 is determined by its bioavailability, which is influenced by various factors, such as food processing, amount of dietary fiber and source of food.[30,31] Moreover, blood vitamin B6 concentration is influenced by the amount of protein in the diet[32] and lifestyle, such as alcohol consumption and smoking status.[33,34] In the present study, the correlation between dietary vitamin B6 intake and serum pyridoxal concentration was low (Spearman correlation coefficient $=0.06$). A few observational studies have reported that vitamin B6 intake and plasma pyridoxal or PLP are weakly or modestly correlated.[33–35] For example, in a study among Puerto Rican adults, the Pearson correlation coefficient between total vitamin B6 intake and fasting plasma PLP was 0.13 among non-users of vitamin B6-containing supplements.[34] The association between vitamin B6 intake and fasting plasma PLP was also not statistically significant among US elderly who did not regularly take vitamin supplements.[35] A study among Dutch without use of supplements observed a modest correlation between dietary vitamin B6 intake and plasma PLP or PLP + pyridoxal (Spearman correlation coefficients: 0.40 and 0.39, respectively, in men and 0.32 and 0.30, respectively, in women).[33] Nevertheless, we observed that both higher serum concentrations and higher dietary intake of vitamin B6 was suggestively associated with a decreased prevalence of depressive symptoms.

Vitamin B6 is a cofactor for enzymes that are essential for the metabolism of amino acids, carbohydrates and fats, and is involved in the synthesis and catabolism of neurotransmitters and transsulfuration of homocysteine.[7] Concerning the link between vitamin B6 and depressive symptoms, the following mechanisms are suggested. First, as vitamin B6 is involved in the one-carbon metabolism pathway and the metabolism of homocysteine to cysteine, vitamin B6 deficiency leads to increased homocysteine concentrations, which has neurotoxic effects[5] and in turn, might result in depressive symptoms.[26] In the present study, however, adjustment for serum homocysteine only slightly attenuated the prospective inverse association between serum pyridoxal and depressive symptoms, and did not change the cross-sectional association. Therefore, the observed association might not be explained by homocysteine pathway. Alternatively, as vitamin B6 is a necessary cofactor in decarboxylation reactions in tryptophan metabolism, it facilitates the conversion of tryptophan to the monoamine neurotransmitter, serotonin.[7]

Major strengths of this study include its prospective design, high study participation rate, measurements of circulating vitamin B6, use of a validated questionnaire for depressive symptoms, and adjustment of known and suspected risk factors of depressive symptoms. As this study was conducted in selected municipal offices at the time of nonselective recruitment for the annual health checkup and had a high study participation rate, the possibility of selection bias is low. In addition, we adjusted for folate and homocysteine, which was reported to be associated with depressive symptoms.[19,20,26,27] Our study also had a few limitations that warrant mention. We measured pyridoxal concentrations, but not those of PLP, which is an active form of vitamin B6 and is commonly used for assessing vitamin B6 status. However, pyridoxal, which is another major form vitamin B6, is strongly correlated with PLP (Spearman correlation coefficients $=0.80$)[36] and may be an appropriate marker of vitamin B6 status.[36,37] Moreover, we measured serum pyridoxal concentrations at only one point in time, which may not reflect long-term status. Serum vitamin B6 concentrations might be affected by inflammation or diabetes associated with depression, and this might account for the observed inverse association. However, the results did not materially change even after excluding subjects with high levels of C-reactive protein, a marker of inflammation, or diabetes. As discussed above, our sample size was not sufficiently large to detect an association between serum pyridoxal and depressive symptoms with statistical significance. Furthermore, few study participants were

taking multivitamin supplements. Therefore, we could not assess the relative merits of supplementation versus dietary intake of vitamin B6. Although we adjusted for risk factors of depressive symptoms, we cannot rule out the possibility of bias because of other confounding factors including socioeconomic status. However, because the study subjects were full-time employees of municipal offices, differences in socioeconomic status across subjects might be small. Finally, the present findings may not be generalizable to a population with a different background.

In conclusion, higher pyridoxal concentrations in serum were associated with decreased prevalence of depressive symptoms, as assessed by the CES-D, independently of folate and homocysteine among Japanese workers. In addition, depressive symptoms after 3 years tended to decrease with increasing levels of serum pyridoxal and dietary vitamin B6 intake at baseline. Our findings suggest that higher vitamin B6 status may prevent the development of depressive symptoms. Further large-scale investigations are required to confirm the present findings and to examine whether improving vitamin B6 status can decrease depressive symptoms.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## ACKNOWLEDGEMENTS

We are grateful to the study participants for their cooperation. We also thank Tamami Hatano, Yasumi Kimura, Akihiro Tanaka, Yuko Ejima, Seiko Miyazaki and Yasutaka Horiuchi (Kyushu University); Mio Ozawa, Emi Tanaka, Youko Tsuruda, Misaki Hirose, Meishu Sai, Miho Isayama, Midori Sasaki, Mie Shimomura and Azumi Uehara (Fukuoka Women's University); Yaeko Nagano (retired nurse); and Akiko Hayashi, Kie Nagao, Yu Teruyama, Kae Saito, Kayoko Washizuka and Yuho Mizoue (National Center for Global Health and Medicine) for their support in data collection. This study was supported by Grant-in-Aid for Young Scientists (B)(21790598) from the Ministry of Education, Culture, Sports, Science and Technology (to Dr Nanri) and Grant-in-Aid for Scientific Research (C)(18590601) and (B)(21390213) from Japan Society for the Promotion of Science (to Dr Mizoue).

## REFERENCES

1 Doris A, Ebmeier K, Shajahan P. Depressive illness. *Lancet* 1999; **354**: 1369–1375.
2 Ministry of Health, Labour and Welfare. *Vital Statistics of Japan*. Health and Welfare Statistics Association: Tokyo, 2008.
3 WHO. Suicide prevention (SUPRE). (cited 2 August 2011); Available at http://www.who.int/mental_health/prevention/suicide/suicideprevent/en/index.html.
4 Reynolds EH. Folic acid, ageing, depression, and dementia. *BMJ* 2002; **324**: 1512–1515.
5 Bottiglieri T. Homocysteine and folate metabolism in depression. *Prog Neuropsychopharmacol Biol Psychiatry* 2005; **29**: 1103–1112.
6 Selhub J. Homocysteine metabolism. *Annu Rev Nutr* 1999; **19**: 217–246.
7 Bernstein AL. Vitamin B6 in clinical neurology. *Ann N Y Acad Sci* 1990; **585**: 250–260.
8 Williams AL, Cotter A, Sabina A, Girard C, Goodman J, Katz DL. The role for vitamin B-6 as treatment for depression: a systematic review. *Fam Pract* 2005; **22**: 532–537.
9 Kamphuis MH, Geerlings MI, Grobbee DE, Kromhout D. Dietary intake of B(6-9-12) vitamins, serum homocysteine levels and their association with depressive symptoms: the Zutphen Elderly Study. *Eur J Clin Nutr* 2008; **62**: 939–945.
10 Merete C, Falcon LM, Tucker KL. Vitamin B6 is associated with depressive symptomatology in Massachusetts elders. *J Am Coll Nutr* 2008; **27**: 421–427.
11 Murakami K, Miyake Y, Sasaki S, Tanaka K, Arakawa M. Dietary folate, riboflavin, vitamin B-6, and vitamin B-12 and depressive symptoms in early adolescence: the Ryukyus Child Health Study. *Psychosom Med* 2010; **72**: 763–768.
12 Murakami K, Mizoue T, Sasaki S, Ohta M, Sato M, Matsushita Y *et al.* Dietary intake of folate, other B vitamins, and omega-3 polyunsaturated fatty acids in relation to depressive symptoms in Japanese adults. *Nutrition* 2008; **24**: 140–147.
13 Sanchez-Villegas A, Doreste J, Schlatter J, Pla J, Bes-Rastrollo M, Martinez-Gonzalez MA. Association between folate, vitamin B(6) and vitamin B(12) intake and depression in the SUN cohort study. *J Hum Nutr Diet* 2009; **22**: 122–133.
14 Tolmunen T, Voutilainen S, Hintikka J, Rissanen T, Tanskanen A, Viinamaki H *et al.* Dietary folate and depressive symptoms are associated in middle-aged Finnish men. *J Nutr* 2003; **133**: 3233–3236.
15 Watanabe H, Ishida S, Konno Y, Matsumoto M, Nomachi S, Masaki K *et al.* Impact of dietary folate intake on depressive symptoms in young women of reproductive age. *J Midwifery Womens Health* 2012; **57**: 43–48.
16 Mishra GD, McNaughton SA, O'Connell MA, Prynne CJ, Kuh D. Intake of B vitamins in childhood and adult life in relation to psychological distress among women in a British birth cohort. *Public Health Nutr* 2009; **12**: 166–174.
17 Skarupski KA, Tangney C, Li H, Ouyang B, Evans DA, Morris MC. Longitudinal association of vitamin B-6, folate, and vitamin B-12 with depressive symptoms among older adults over time. *Am J Clin Nutr* 2010; **92**: 330–335.
18 Hvas AM, Juul S, Bech P, Nexo E. Vitamin B6 level is associated with symptoms of depression. *Psychother Psychosom* 2004; **73**: 340–343.
19 Nanri A, Hayabuchi H, Ohta M, Sato M, Mishima N, Mizoue T. Serum folate and depressive symptoms among Japanese men and women: a cross-sectional and prospective study. *Psychiatry Res* 2012; **200**: 349–353.
20 Nanri A, Mizoue T, Matsushita Y, Sasaki S, Ohta M, Sato M *et al.* Serum folate and homocysteine and depressive symptoms among Japanese men and women. *Eur J Clin Nutr* 2010; **64**: 289–296.
21 Shima S, Shikano T, Kitamura T, Asai M. New self-rating scale for Depression. *Jpn J Clin Psychiatry* 1985; **27**: 717–723 (in Japanese).
22 Radloff LS. The CES-D scale: a self-report depression scale for research in the general population. *Appl Psychol Meas* 1977; **1**: 385–401.
23 Wada K, Tanaka K, Theriault G, Satoh T, Mimura M, Miyaoka H *et al.* Validity of the Center for Epidemiologic Studies Depression Scale as a screening instrument of major depressive disorder among Japanese workers. *Am J Ind Med* 2007; **50**: 8–12.
24 Kobayashi S, Honda S, Murakami K, Sasaki S, Okubo H, Hirota N *et al.* Both comprehensive and brief self-administered diet history questionnaires satisfactorily rank nutrient intakes in Japanese adults. *J Epidemiol* 2012; **22**: 151–159.
25 Science and Technology Agency. *Standard Tables of Food Composition in Japan*. 5th revised and enlarged ed. Printing Bureau of the Ministry of Finance: Tokyo 2005.
26 Bjelland I, Tell GS, Vollset SE, Refsum H, Ueland PM. Folate, vitamin B12, homocysteine, and the MTHFR 677C->T polymorphism in anxiety and depression: the Hordaland Homocysteine Study. *Arch Gen Psychiatry* 2003; **60**: 618–626.
27 Gilbody S, Lightfoot T, Sheldon T. Is low folate a risk factor for depression? A meta-analysis and exploration of heterogeneity. *J Epidemiol Community Health* 2007; **61**: 631–637.
28 Yi S, Nanri A, Poudel-Tandukar K, Nonaka D, Matsushita Y, Hori A *et al.* Association between serum ferritin concentrations and depressive symptoms in Japanese municipal employees. *Psychiatry Res* 2011; **189**: 368–372.
29 Ministry of Health, Labour and Welfare. *The National Health and Nutrition Survey in Japan, 2008*. Daiichi-shuppan: Tokyo, 2011.
30 Reynolds RD. Bioavailability of vitamin B-6 from plant foods. *Am J Clin Nutr* 1988; **48**: 863–867.
31 Gregory 3rd JF, Kirk JR. The bioavailability of vitamin B6 in foods. *Nutr Rev* 1981; **39**: 1–8.
32 Miller LT, Leklem JE, Shultz TD. The effect of dietary protein on the metabolism of vitamin B-6 in humans. *J Nutr* 1985; **115**: 1663–1672.
33 Brussaard JH, Lowik MR, van den Berg H, Brants HA, Bemelmans W. Dietary and other determinants of vitamin B6 parameters. *Eur J Clin Nutr* 1997; **51**(Suppl 3): S39–S45.
34 Ye X, Maras JE, Bakun PJ, Tucker KL. Dietary intake of vitamin B-6, plasma pyridoxal 5'-phosphate, and homocysteine in Puerto Rican adults. *J Am Diet Assoc* 2010; **110**: 1660–1668.
35 Manore MM, Vaughan LA, Carroll SS, Leklem JE. Plasma pyridoxal 5'-phosphate concentration and dietary vitamin B-6 intake in free-living, low-income elderly people. *Am J Clin Nutr* 1989; **50**: 339–345.
36 Midttun O, Hustad S, Schneede J, Vollset SE, Ueland PM. Plasma vitamin B-6 forms and their relation to transsulfuration metabolites in a large, population-based study. *Am J Clin Nutr* 2007; **86**: 131–138.
37 Leklem JE. Vitamin B-6: a status report. *J Nutr* 1990; **120**(Suppl 11): 1503–1507.