

Dietary intake of folate, vitamin B₆, vitamin B₁₂ and riboflavin and risk of Parkinson's disease: a case–control study in Japan

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Increased homocysteine levels might accelerate dopaminergic cell death in Parkinson's disease (PD) through neurotoxic effects; thus, increasing intake of B vitamins involved in the regulation of homocysteine metabolism might decrease the risk of PD through decreasing plasma homocysteine. However, epidemiological evidence for the association of dietary B vitamins with PD is sparse, particularly in non-Western populations. We conducted a hospital-based case–control study in Japan to examine associations between dietary intake of folate, vitamin B₆, vitamin B₁₂ and riboflavin and the risk of PD. Patients with PD diagnosed using the UK PD Society Brain Bank criteria (*n* 249) and controls without neurodegenerative diseases (*n* 368) were recruited. Dietary intake during the preceding month was assessed at the time of study recruitment using a validated, self-administered, semi-quantitative, comprehensive diet history questionnaire. After adjustment for potential dietary and non-dietary confounding factors, intake of folate, vitamin B₁₂ and riboflavin was not associated with the risk of PD (*P* for trend=0.87, 0.70 and 0.11, respectively). However, low intake of vitamin B₆ was associated with an increased risk of PD, independent of potential dietary and non-dietary confounders. Multivariate OR (95% CI) for PD in the first, second, third and fourth quartiles of vitamin B₆ were 1 (reference), 0.56 (0.33, 0.94), 0.69 (0.38, 1.25) and 0.48 (0.23, 0.99), respectively (*P* for trend=0.10). In conclusion, in the present case–control study in Japan, low intake of vitamin B₆, but not of folate, vitamin B₁₂ or riboflavin, was independently associated with an increased risk of PD.

Diet: Folate: Vitamin B₆: Vitamin B₁₂: Parkinson's disease

Parkinson's disease (PD), which is characterised by bradykinesia, rigidity with cogwheeling, rest tremor and postural instability, is one of the most common neurodegenerative disorders. Although genetic factors are thought to play an important role in some cases of PD, environmental and lifestyle factors are probably responsible for the majority of cases⁽¹⁾. Although symptomatic treatment has improved in recent years, a cure for PD is not yet available. Because

the cause of onset is largely unknown, epidemiology is important in defining the cause of PD and in evaluating preventive measures.

The importance of research on the possible role of dietary factors in PD is emphasised by the fact that diet is modifiable. Given that increased homocysteine levels might accelerate dopaminergic cell death in PD through neurotoxic effects⁽²⁾, increasing intake of B vitamins involved in the

Abbreviations: DHQ, diet history questionnaire; PD, Parkinson's disease.

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regulation of homocysteine metabolism (i.e. folate, vitamin B₆, vitamin B₁₂ and riboflavin) might decrease the risk of PD through decreasing plasma homocysteine^(3,4). However, epidemiological evidence for the association of intake of these B vitamins with PD is sparse^(5–8), and no studies have been conducted in non-Western populations.

Here, we examined the association between dietary intake of folate, vitamin B₆, vitamin B₁₂ and riboflavin and the risk of PD using data from a multicentre hospital-based case–control study in Japan^(9,10). Population-specific genetic characteristics (e.g. single-nucleotide polymorphisms influencing homocysteine metabolism)⁽¹¹⁾ especially explain the need to investigate this topic in a Japanese population.

Subjects and methods

Study population

The present study was based on a multicentre hospital-based case–control study conducted at eleven collaborating hospitals in Japan (three university and one national hospitals in Fukuoka, and three university, three national and one municipal hospitals in Kinki). Recruitment of patients with PD was done at all the collaborating hospitals. Eligible cases were patients who were within 6 years of the onset of PD, and had received treatment at one of the collaborating hospitals during the period 1 April 2006 to 31 March 2008. PD was diagnosed by the collaborating neurologists using the UK PD Society Brain Bank criteria⁽¹²⁾. The neurologists in charge invited 298 eligible PD patients to take part; of these, 250 patients were cooperative in answering the questionnaires, whereas 48 declined (response rate: 84 %).

Recruitment of control subjects was done at three of the eleven collaborating hospitals (one university hospital in Fukuoka, and one university and one national hospitals in Kinki). Eligible control subjects were inpatients and outpatients without neurodegenerative diseases (i.e. orthopaedic surgery, ophthalmology, otorhinolaryngology, plastic surgery and oral surgery) during the period 1 April 2006 to 31 March 2008. Controls were not matched to cases either individually or by group. A total of 528 patients were approached by the attending doctors or our research nurses for recruitment as controls; of whom, 372 agreed, while 156 declined (response rate: 70 %).

For analysis, one case and four controls were excluded due to missing data on the factors under study, leaving 249 cases and 368 controls for the final analysis. The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects/patients were approved by the ethics committees of the eleven collaborating hospitals (Faculty of Medicine, Fukuoka University; Utano National Hospital; Osaka City University Graduate School of Medicine; Graduate School of Medical Sciences, Kyushu University; Wakayama Medical University; Kyoto University Graduate School of Medicine; Kurume University School of Medicine; Minami-Kyoto National Hospital; Toneyama National Hospital; Kyoto City Hospital and National Omuta Hospital). Written informed consent was obtained from all subjects/patients.

Measurements

Case and control subjects filled out a set of two self-administered questionnaires, and mailed them to the data management centre or handed them to research nurses. Research technicians completed missing or illogical data by telephone or direct interview.

Dietary habits during the preceding month were assessed using a self-administered, semi-quantitative, comprehensive, diet history questionnaire (DHQ). Details of the DHQ's structure and calculation of dietary intake, and validity for commonly studied nutritional factors have been published elsewhere^(13–16). Briefly, the DHQ is a structured sixteen-page questionnaire which asks about the consumption frequency and portion size of selected foods commonly consumed in Japan as well as about general dietary behaviour and usual cooking methods^(13,16). Estimates of daily intake for foods (150 items in total), energy and selected nutrients were calculated using an *ad hoc* computer algorithm for the DHQ^(13,16) based on the Standard Tables of Food Composition in Japan^(17,18). Because only a small number of subjects used dietary supplements weekly or more often (e.g. vitamin B complex, 4 % and multivitamins, 7 %), the use of dietary supplements was not incorporated into the analysis. Dietary glycaemic index, a measure of carbohydrate quality, was calculated according to a procedure described elsewhere^(10,16,19). To minimise the influence of dietary misreporting, an ongoing controversy in studies that collect dietary information using self-reported instruments⁽²⁰⁾, dietary variables were energy adjusted using the density method (except for glycaemic index)^(10,16,19). In a previous study of forty-seven Japanese women, Pearson's correlation coefficients between the DHQ and 3 d estimated dietary records were 0.48 for energy, 0.37–0.75 for energy-providing nutrients and 0.38–0.68 for other nutrients⁽¹³⁾; in another study of ninety-two Japanese women and ninety-two Japanese men⁽¹⁶⁾, Pearson's correlation coefficients between the DHQ and 16 d weighed dietary records were 0.58 and 0.39 for folate, 0.63 and 0.58 for vitamin B₆, 0.51 and 0.41 for vitamin B₁₂, 0.51 and 0.39 for riboflavin, 0.42 and 0.49 for cholesterol, 0.50 and 0.58 for dietary glycaemic index, 0.42 and 0.48 for vitamin E, 0.45 and 0.47 for vitamin C, 0.63 and 0.40 for β -carotene, 0.74 and 0.82 for alcohol, 0.43 and 0.38 for caffeine, and 0.68 and 0.52 for Fe, respectively (Sasaki S, unpublished results, 2006), suggesting satisfactory validity of the DHQ in terms of B vitamins and other dietary variables.

Body weight and height were self-reported as part of the DHQ. BMI was calculated as weight (kg) divided by the square of height (m²). The second questionnaire on non-dietary factors potentially associated with PD elicited information on sex, age, education and smoking habits. This questionnaire was developed for this survey based on comprehensive literature review of epidemiological studies on risk factors for PD, although its validity has not been investigated.

Statistical analysis

All statistical analyses were performed using SAS statistical software version 9.1 (SAS Institute, Inc., Cary, NC, USA). Dietary intake (amount per 4184 kJ of energy) of B vitamins

(i.e. folate, vitamin B₆, vitamin B₁₂ and riboflavin) was categorised at quartile points on the distribution of control subjects. Using logistic regression analysis, crude and multivariate-adjusted OR and 95 % CI for PD for each quartile category of dietary intake were calculated (i.e. models 1 and 2). The lowest quartile category of dietary intake was used as a reference category. Multivariate-adjusted OR (model 2) were calculated by adjusting for potential non-dietary confounding factors^(9,10), including sex (men or women), age (years, continuous), region (Fukuoka or Kinki), pack-years of smoking (none, 0.1–29.9 or ≥30.0), education (<10, 10–12 or ≥12 years) and BMI (kg/m², continuous). Further adjustment (i.e. model 3) was done for potential dietary confounding factors (continuous), i.e. variables associated with PD in this population^(9,10), namely cholesterol (mg/4184 kJ) and glycaemic index, and variables possibly associated with PD according to the literature^(1,4), namely vitamin E (mg α-tocopherol/4184 kJ), vitamin C (mg/4184 kJ), β-carotene (μg/4184 kJ), alcohol (% of energy), caffeine (mg/4184 kJ) and Fe (mg/4184 kJ). Finally, additional adjustment for intake of other B vitamins (continuous) was done (i.e. model 4). We also conducted four sensitivity analyses (using model 4): that confined to cases diagnosed less than 3 years from onset; that excluding control subjects having diseases of the digestive tract; that confined to subjects recruited from three of the eleven collaborating hospitals at which cases were recruited; and that excluding dietary supplement users.

Trends of association were assessed by a logistic regression model which assigned consecutive integers to the levels of the independent variable. All reported *P* values are two-tailed, and *P* values <0.05 were considered statistically significant.

Results

Characteristics of the study population are shown in Table 1. About 40 % of both case and control subjects were males, and recruited from the Fukuoka region. Cases were more likely to be older, have lower BMI and be non-smokers. Cases also had a significantly lower intake of folate, alcohol and caffeine, and a significantly higher intake of cholesterol. There were no differences between cases and controls in education and other dietary variables, or in sex or region.

Table 2 shows sample characteristics of the lowest and highest quartiles of dietary intake of B vitamins. There were more women and non-smokers in higher quartiles of dietary folate, vitamin B₆ and riboflavin. There was a positive association between intake of folate and riboflavin and age. Vitamin B₆ intake was inversely associated with BMI. There were positive associations among intake of B vitamins examined; Pearson's correlation coefficients ranged from 0.39 (folate and vitamin B₁₂) to 0.68 (folate and riboflavin). Intake of B vitamins was associated positively with intake of cholesterol, vitamins E and C, β-carotene and caffeine, and inversely with dietary glycaemic index and alcohol.

Table 1. Characteristics of the study population (Mean values and standard deviations)

	Parkinson's disease				<i>P</i> *
	Cases (n 249)		Controls (n 368)		
	Mean	SD	Mean	SD	
Male sex (%)		37.4		38.3	0.81
Age (years)	68.5	8.6	66.6	8.5	0.006
Region (%)					0.13
Fukuoka		35.7		41.9	
Kinki		64.3		58.2	
Pack-years of smoking (%)					0.0004
None		74.3		60.3	
0.1–29.9		14.9		17.7	
≥30.0		10.8		22.0	
Education (%)					0.81
<10 years		20.5		20.9	
10–12 years		49.0		46.5	
≥13 years		30.5		32.6	
BMI (kg/m ²)	22.3	3.3	23.0	3.4	0.01
Dietary intake					
Energy (kJ/d)	8765	2636	8665	3067	0.71
Folate (μg/4184 kJ)	163	47	173	57	0.02
Vitamin B ₆ (mg/4184 kJ)	0.63	0.18	0.64	0.18	0.32
Vitamin B ₁₂ (μg/4184 kJ)	4.2	2.3	4.1	2.2	0.66
Riboflavin (mg/4184 kJ)	0.69	0.19	0.71	0.22	0.35
Cholesterol (mg/4184 kJ)	159	62	149	62	0.04
Dietary glycaemic index	65.1	4.7	65.4	5.3	0.40
Vitamin E (mg α-tocopherol/4184 kJ)	4.2	1.2	4.2	1.2	0.93
Vitamin C (mg/4184 kJ)	62.7	30.3	60.3	30.6	0.33
β-Carotene (μg/4184 kJ)	1499	820	1608	949	0.14
Alcohol (% of energy)	1.8	4.6	2.9	7.3	0.04
Caffeine (mg/4184 kJ)	149	109	195	138	<0.0001
Fe (mg/4184 kJ)	3.7	0.9	3.8	1.0	0.12

* For categorical variables, the χ^2 test was used; for continuous variables, the independent-samples *t* test was used.

Table 2. Sample characteristics for the lowest (Q1) and highest (Q4) quartiles of dietary intake of B vitamins
(Mean values and percentages)

	Folate			Vitamin B ₆			Vitamin B ₁₂			Riboflavin		
	Q1	Q4	P*	Q1	Q4	P*	Q1	Q4	P*	Q1	Q4	P*
<i>n</i>	165	138		169	144		155	163		157	146	
Male sex (%)	60.0	17.4	<0.0001	50.3	34.0	0.0004	40.7	34.4	0.69	54.8	23.3	<0.0001
Age (years)	65.5	68.2	0.004	66.6	67.4	0.62	65.9	67.9	0.27	66.1	68.6	0.006
Region (%)			0.01			0.06			0.17			0.13
Fukuoka	32.7	50.7		34.3	48.6		41.9	36.8		33.1	44.5	
Kinki	67.3	49.3		65.7	51.4		58.1	63.2		66.9	55.5	
Pack-years of smoking (%)			<0.0001			0.01			0.20			<0.0001
None	47.9	81.9		55.0	75.0		60.7	69.3		49.0	77.4	
0.1–29.9	25.5	7.3		21.9	10.4		21.9	10.4		23.6	11.0	
≥30.0	26.7	10.9		23.1	14.6		17.4	20.3		27.4	11.6	
Education (%)			0.62			0.40			0.46			0.77
<10 years	23.6	17.4		21.3	15.3		21.3	19.6		21.0	17.8	
10–12 years	44.9	47.8		50.3	47.9		49.7	47.9		48.4	51.4	
≥13 years	31.5	34.8		28.4	36.8		29.0	32.5		30.6	30.8	
BMI (kg/m ²)	23.0	22.8	0.59	23.3	22.6	0.03	23.2	22.5	0.13	23.0	22.8	0.32
Dietary intake												
Energy (kJ/d)	8839	8066	0.02	8247	8766	0.02	8100	9205	<0.0001	8489	8432	0.69
Folate (μg/4184 kJ)	109	246	–	126	210	<0.0001	140	195	<0.0001	124	216	<0.0001
Vitamin B ₆ (mg/4184 kJ)	0.49	0.80	<0.0001	0.43	0.88	–	0.51	0.77	<0.0001	0.52	0.73	<0.0001
Vitamin B ₁₂ (μg/4184 kJ)	3.0	5.3	<0.0001	2.6	5.7	<0.0001	1.9	7.1	–	2.9	5.4	<0.0001
Riboflavin (mg/4184 kJ)	0.53	0.88	<0.0001	0.57	0.82	<0.0001	0.58	0.82	<0.0001	0.46	0.98	–
Cholesterol (mg/4184 kJ)	128	168	<0.0001	124	171	<0.0001	114	192	<0.0001	110	187	<0.0001
Dietary glycaemic index	67.0	63.0	<0.0001	67.9	61.8	<0.0001	66.4	64.0	<0.0001	67.2	63.3	<0.0001
Vitamin E (mg α-tocopherol/4184 kJ)	3.3	5.1	<0.0001	3.2	5.1	<0.0001	3.5	4.8	<0.0001	3.5	4.6	<0.0001
Vitamin C (mg/4184 kJ)	39.2	91.3	<0.0001	42.9	79.5	<0.0001	50.8	69.1	<0.0001	40.4	84.1	<0.0001
β-Carotene (μg/4184 kJ)	945	2323	<0.0001	1060	2056	<0.0001	1294	1758	<0.0001	1213	1819	<0.0001
Alcohol (% of energy)	5.6	0.9	<0.0001	3.8	1.6	0.01	3.3	2.2	0.21	5.7	1.3	<0.0001
Caffeine (mg/4184 kJ)	116	243	<0.0001	164	197	0.02	165	169	0.67	109	260	<0.0001
Fe (mg/4184 kJ)	2.9	4.8	<0.0001	3.0	4.6	<0.0001	3.2	4.4	<0.0001	3.0	4.5	<0.0001

* For categorical variables, the χ^2 test was used; for continuous variables, a linear trend test was used.

Table 3. OR for Parkinson's disease according to the quartile of dietary intake of B vitamins (Odds ratios and 95 % confidence intervals)

	Quartile 1 (lowest)	Quartile 2		Quartile 3		Quartile 4 (highest)		P for trend
	OR (reference)	OR	95 % CI	OR	95 % CI	OR	95 % CI	
Folate ($\mu\text{g}/4184 \text{ kJ}$)*	113 (37–137)	152 (138–165)		182 (166–204)		237 (205–456)		
<i>n</i> of cases and controls	73/92	71/92		59/92		46/92		
Model 1†	1	0.97	0.63, 1.50	0.81	0.52, 1.27	0.63	0.39, 1.01	0.04
Model 2‡	1	0.81	0.51, 1.29	0.65	0.40, 1.06	0.50	0.30, 0.85	0.007
Model 3§	1	0.93	0.54, 1.60	0.89	0.47, 1.70	0.83	0.34, 2.01	0.68
Model 4	1	1.00	0.57, 1.74	0.97	0.50, 1.86	0.93	0.38, 2.31	0.87
Vitamin B₆ (mg/4184 kJ)*	0.45 (0.14–0.52)	0.58 (0.53–0.62)		0.68 (0.63–0.74)		0.84 (0.75–1.37)		
<i>n</i> of cases and controls	77/92	59/92		61/92		52/92		
Model 1†	1	0.77	0.49, 1.20	0.79	0.51, 1.23	0.68	0.43, 1.07	0.11
Model 2‡	1	0.64	0.40, 1.03	0.75	0.47, 1.19	0.58	0.36, 0.94	0.052
Model 3§	1	0.57	0.34, 0.96	0.71	0.40, 1.26	0.53	0.26, 1.05	0.13
Model 4	1	0.56	0.33, 0.94	0.69	0.38, 1.25	0.48	0.23, 0.99	0.10
Vitamin B₁₂ ($\mu\text{g}/4184 \text{ kJ}$)*	2.0 (0.3–2.5)	3.2 (2.6–3.6)		4.3 (3.7–5.1)		6.6 (5.2–19.9)		
<i>n</i> of cases and controls	63/92	69/92		46/92		71/92		
Model 1†	1	1.10	0.70, 1.71	0.73	0.45, 1.18	0.13	0.72, 1.76	0.98
Model 2‡	1	0.95	0.59, 1.51	0.68	0.41, 1.11	1.03	0.65, 1.65	0.82
Model 3§	1	0.98	0.60, 1.62	0.72	0.42, 1.24	1.10	0.61, 1.98	0.93
Model 4	1	1.05	0.63, 1.74	0.79	0.45, 1.38	1.29	0.69, 2.44	0.70
Riboflavin (mg/4184 kJ)*	0.48 (0.13–0.57)	0.64 (0.58–0.70)		0.76 (0.71–0.84)		0.95 (0.85–1.48)		
<i>n</i> of cases and controls	65/92	75/92		55/92		54/92		
Model 1†	1	1.15	0.74, 1.79	0.85	0.53, 1.34	0.83	0.52, 1.32	0.24
Model 2‡	1	1.05	0.66, 1.66	0.69	0.42, 1.12	0.69	0.42, 1.13	0.06
Model 3§	1	0.92	0.55, 1.55	0.65	0.35, 1.20	0.62	0.29, 1.32	0.13
Model 4	1	0.90	0.53, 1.54	0.64	0.34, 1.20	0.58	0.26, 1.28	0.11

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* Values are median (range).

† Crude model.

‡ Adjusted for non-dietary factors, including sex (men or women), age (years, continuous), region (Fukuoka or Kinki), pack-years of smoking (none, 0.1–29.9 or ≥ 30.0), education (<10, 10–12 or ≥ 12 years) and BMI (kg/m^2 , continuous).

§ Adjusted for non-dietary factors (variables used in model 2) and dietary factors (continuous), including cholesterol (mg/4184 kJ), dietary glycaemic index, vitamin E (mg α -tocopherol/4184 kJ), vitamin C (mg/4184 kJ), β -carotene ($\mu\text{g}/4184 \text{ kJ}$), alcohol (% of energy), caffeine (mg/4184 kJ) and Fe (mg/4184 kJ).

|| Adjusted for non-dietary and dietary factors (variables used in model 3) and intake of other B vitamins (continuous), including folate ($\mu\text{g}/4184 \text{ kJ}$), vitamin B₆ (mg/4184 kJ), vitamin B₁₂ ($\mu\text{g}/4184 \text{ kJ}$) and riboflavin (mg/4184 kJ).

Table 3 shows crude and multivariate OR for PD according to the quartile of dietary intake of B vitamins. After adjustment for potential non-dietary confounding factors (model 2), dietary intake of folate was inversely associated with the risk of PD. Multivariate OR for PD in the first, second, third and fourth quartiles of dietary folate were 1 (reference), 0.81 (95% CI 0.51, 1.29), 0.65 (95% CI 0.40, 1.06) and 0.50 (95% CI 0.30, 0.85), respectively (P for trend=0.007). However, the association between folate and PD disappeared after further adjustment for potential dietary confounding factors (model 3; P for trend=0.68) and other B vitamins (model 4; P for trend=0.87).

Conversely, low intake of vitamin B₆ was associated with an increased risk of PD, independent of non-dietary confounding factors (model 2). Multivariate OR for PD in the first, second, third and fourth quartiles of dietary vitamin B₆ were 1 (reference), 0.64 (95% CI 0.40, 1.03), 0.75 (95% CI 0.47, 1.19) and 0.58 (95% CI 0.36, 0.94), respectively (P for trend=0.052). This association between vitamin B₆ and PD was generally retained after further adjustment for dietary confounders (model 3; P for trend=0.13) and other B vitamins (model 4). Multivariate OR for PD in the first, second, third and fourth quartiles of dietary vitamin B₆ were 1 (reference), 0.56 (95% CI 0.33, 0.94), 0.69 (95% CI 0.38, 1.25) and 0.48 (95% CI 0.23, 0.99), respectively (P for trend=0.10). Intake of vitamin B₁₂ and riboflavin was not associated with the risk of PD.

Discussion

In the present case-control study in Japan, we found that after adjustment for potential dietary and non-dietary confounding factors, low intake of dietary vitamin B₆ was associated with an increased risk of PD. In contrast, no independent association was seen between intake of folate, vitamin B₁₂ and riboflavin and the risk of PD. To our knowledge, this is the first study to examine the relationship of dietary B vitamins with PD in non-Western populations.

A limited number of epidemiological studies have examined this topic. A case-control study in Germany found that a higher intake of folate, vitamin B₆ and vitamin B₁₂, but not of riboflavin, was associated with a lower risk of PD⁽⁵⁾. A prospective cohort study in the Netherlands showed that a higher intake of vitamin B₆, but not of folate or vitamin B₁₂, was associated with a decreasing risk of PD⁽⁶⁾. These are partially consistent with the present findings. Conversely, a case-control study in the US found no association for intake of folate, vitamin B₆ or vitamin B₁₂⁽⁷⁾, while a large US-based prospective study in health professionals found no association between intake of folate, vitamin B₆ or vitamin B₁₂ and PD risk⁽⁸⁾. Considering the presence of food fortification with folic acid, however, the USA may not be the ideal setting to assess this association⁽¹⁾. These discrepancies among studies may be explained, at least in part, by differences in study population and design, dietary assessment methods used, definitions of PD applied and potential confounding factors considered.

The association between the intake of nutrients that influence homocysteine concentration and the risk of PD can be explained by homocysteine metabolism because of the potential neurotoxic effects of homocysteine⁽¹⁾. Previous studies

have generally shown that dietary intake of folate and related B vitamins such as vitamin B₆ and riboflavin is moderately associated with plasma homocysteine concentration, whereas no such association is expected for vitamin B₁₂, at least when folate intake is within a normal range^(21–25). The findings are generally inconsistent with the present result that low intake of vitamin B₆, but not of folate, vitamin B₁₂ or riboflavin, is associated with an increased risk of PD. The association between vitamin B₆ and PD might be explained by neuroprotective properties of vitamin B₆ through antioxidant capacities (unrelated to homocysteine metabolism)^(26,27), and through its role in dopamine synthesis⁽²⁸⁾. Alternatively, vitamin B₆ intake might be a marker of dietary patterns that are related to decreased PD risk (e.g. high intake of a wide range of antioxidants). Additionally, a potential effect of blood levels of B vitamins involved in the regulation of homocysteine metabolism is not ruled out by the present study based on dietary intake. Thus, future research which directly examines the association of PD with blood concentrations of homocysteine and related B vitamins would be of interest.

Several limitations of the study warrant mention. First, diagnosis of PD is based on clinical symptoms, and it is assumed that some proportion of cases may be misdiagnosed, which could cause bias towards the null. Nevertheless, cases were identified according to strict diagnostic criteria, and thus the possibility of misclassification of PD is small.

One may speculate that data obtained from PD patients based on the two self-administered questionnaires are not reliable on the basis that PD affects several aspects of symptoms including movement, mood, behaviour, thinking and sensation. Nevertheless, responses to the questionnaires were carefully checked for completeness by research technicians trained for this survey, who clarified any missing values or errors of logic by telephone or direct interview with subjects. The possible influence of the symptoms of PD on the quality of data derived from the questionnaires should thus have been minimised.

The dietary assessment questionnaire used (i.e. DHQ) has not been validated in the present population, although it has been validated in other Japanese groups^(13–16). The possibility of misclassification might have biased the magnitude of the observed associations towards the null. More importantly, current rather than past dietary habits were assessed, not only because the critical period of life during which PD develops is the subject of much controversy^(29,30) but also because retrospective estimation of past diet is generally difficult⁽³¹⁾, and the utility of our DHQ for past diet is unknown. Thus, the present study is inevitably based on the assumption that the relative food intake patterns of cases and controls have remained fairly stable over time. Nevertheless, this may be reasonable given that there seem to be no special diets for PD followed in Japan, albeit that dopamine shortage can affect food preferences⁽³²⁾. In this regard, the results of a sensitivity analysis confined to cases diagnosed less than 3 years from onset (n 109) were similar to those of the overall analysis, with the exception of a positive association for vitamin B₁₂. The multivariate OR in the highest (compared with the lowest) quartile were 0.51 (95% CI 0.16, 1.66) for folate (P for trend=0.20), 0.30 (95% CI 0.11, 0.82) for vitamin B₆ (P for trend=0.0496), 2.55 (95% CI 1.10, 5.92) for vitamin B₁₂ (P for trend=0.09) and 0.49 (95% CI 0.17, 1.37) for riboflavin (P for trend=0.19).

Because our controls were not normal healthy persons, but rather inpatients and outpatients without neurodegenerative diseases, they may be unlikely to provide an estimate of the exposure prevalence in the population from which the cases arose, although the response rate was relatively high (70%). In Japan at least, the use of normal healthy people in the community as controls is not always sufficient, given the relatively low response rate observed in a case-control study in Japan (50%)⁽³³⁾. Some control subjects had diseases of the digestive tract such as stomach cancer ($n = 2$), or diseases associated with long-term modification of the diet such as diabetes ($n = 42$; no controls with ulcerative colitis or Crohn's disease). Nevertheless, the results of a sensitivity analysis excluding these subjects were similar to those of the overall analysis, with the exception of no association for vitamin B₆. The multivariate OR in the highest (compared with the lowest) quartile were 1.03 (95% CI 0.41, 2.58) for folate (P for trend=0.94), 0.53 (95% CI 0.25, 1.11) for vitamin B₆ (P for trend=0.15), 1.15 (95% CI 0.60, 2.22) for vitamin B₁₂ (P for trend=0.97) and 0.59 (95% CI 0.26, 1.33) for riboflavin (P for trend=0.12). Furthermore, although our control subjects were selected from three of the eleven collaborating hospitals at which cases were recruited, the results of a sensitivity analysis confined to patients recruited from these three hospitals ($n = 153$) were similar to those of the overall analysis, with the exception of no association for vitamin B₆. The multivariate OR in the highest (compared with the lowest) quartile were 0.65 (95% CI 0.23, 1.81) for folate (P for trend=0.53), 0.68 (95% CI 0.30, 1.55) for vitamin B₆ (P for trend=0.50), 1.94 (95% CI 0.93, 4.06) for vitamin B₁₂ (P for trend=0.12) and 0.57 (95% CI 0.23, 1.41) for riboflavin (P for trend=0.17).

As mentioned above, the percentage of subjects using dietary supplements such as vitamin B complex and multivitamins in the present study was not large (12% ($n = 29$) for cases and 10% ($n = 38$) for controls). Moreover, the exclusion of these dietary supplement users from the analysis did not materially alter the results. The multivariate OR in the highest (compared with the lowest) quartile were 1.06 (95% CI 0.41, 2.77) for folate (P for trend=0.81), 0.45 (95% CI 0.21, 0.98) for vitamin B₆ (P for trend=0.12), 1.09 (95% CI 0.56, 2.15) for vitamin B₁₂ (P for trend=0.93) and 0.58 (95% CI 0.25, 1.38) for riboflavin (P for trend=0.13). Thus, it is unlikely that dietary supplements had a major effect on the present findings.

Although adjustments to compensate for a variety of potential confounding variables were attempted, residual confounding effects could not be ruled out. In particular, potential non-dietary confounding factors were assessed using a non-validated questionnaire. Finally, while information on cognitive impairment is unfortunately unavailable in the present study, there may be some subjects (particularly PD patients) with cognitive impairment, which can affect both dietary intake and the ability to recall diet, and hence the present results.

In conclusion, the present case-control study in Japan showed that low intake of vitamin B₆, but not of folate, vitamin B₁₂ or riboflavin, was independently associated with an increased risk of PD. Because epidemiological research on this topic is still sparse, further evidence from well-designed case-control and prospective cohort studies is required to

accumulate evidence on potential beneficial role of dietary B vitamins in the development of PD.

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Appendix

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