



## Folate Intake and Risk of Parkinson's Disease

Honglei Chen<sup>1</sup>, Shumin M. Zhang<sup>2,3</sup>, Michael A. Schwarzschild<sup>4</sup>, Miguel A. Hernán<sup>2</sup>,  
Giancarlo Logroscino<sup>2</sup>, Walter C. Willett<sup>1,2,5</sup>, and Alberto Ascherio<sup>1,2,5</sup>

<sup>1</sup> Department of Nutrition, Harvard School of Public Health, Boston, MA.

<sup>2</sup> Department of Epidemiology, Harvard School of Public Health, Boston, MA.

<sup>3</sup> Division of Preventive Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

<sup>4</sup> Department of Neurology, Massachusetts General Hospital, Boston, MA.

<sup>5</sup> Channing Laboratory, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.

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In clinical studies, individuals with Parkinson's disease have had higher concentrations of plasma homocysteine than did controls, and experimental evidence suggests that folate deficiency or focal administration of homocysteine sensitizes dopaminergic neurons to the neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. The authors thus prospectively investigated whether higher intake of folate, vitamin B<sub>6</sub>, or vitamin B<sub>12</sub> was related to a lower risk of Parkinson's disease in the Health Professionals Follow-up Study (1986–2000) and the Nurses' Health Study (1980–1998). They documented Parkinson's disease diagnoses in 248 men and 167 women during the follow-up. Folate intake was not associated with the risk of Parkinson's disease; the relative risks for the highest compared with the lowest quintiles were 1.0 (95% confidence interval: 0.7, 1.5) in men and 1.3 (95% confidence interval: 0.8, 2.3) in women. Neither did they find significant associations in analyses stratified by age, smoking, alcohol consumption, or lactose intake. Intake of vitamin B<sub>6</sub> or vitamin B<sub>12</sub> also was not related to the risk of Parkinson's disease. The current study does not support the hypothesis that higher intake of folate or related B vitamins lowers the risk of Parkinson's disease.

cohort studies; diet; folic acid; homocysteine; Parkinson disease

Abbreviations: CI, confidence interval; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

Low folate intake increases plasma homocysteine (1), which damages the vascular endothelium and increases the risk of cardiovascular diseases (2). Homocysteine is also neurotoxic, and hyperhomocysteinemia has been associated prospectively with higher risk of Alzheimer's disease in the Framingham Study (3). A higher plasma homocysteine concentration has also been reported in Parkinson's disease patients than in controls (4–6), but this elevation could be a consequence rather than a cause of Parkinson's disease. The potential neurotoxicity of homocysteine to dopaminergic neurons was recently investigated in an animal model of Parkinson's disease, in which a folate-deficient diet or direct administration of homocysteine significantly enhanced the neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (7). The results suggest that folate deficiency

and hyperhomocysteinemia may potentially contribute to Parkinson's disease pathogenesis. Therefore, we prospectively investigated whether intake of folate or of related B vitamins that are involved in folate and homocysteine metabolism was associated with Parkinson's disease risk in two large ongoing prospective cohorts: the Health Professionals Follow-up Study and the Nurses' Health Study.

### MATERIALS AND METHODS

#### Study population

The Health Professionals Follow-up Study cohort was established in 1986, when 51,529 male health professionals (dentists, optometrists, pharmacists, osteopaths, podiatrists,

Correspondence to Dr. Honglei Chen, Department of Nutrition, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115 (e-mail: hchen@hsph.harvard.edu).

and veterinarians), aged 40–75 years, responded to a mailed questionnaire that included a 131-item food frequency questionnaire (8), in addition to questions on history of diseases and lifestyle. The Nurses' Health Study was established in 1976 when 121,700 registered nurses aged 30–55 years in 11 states provided detailed information about their medical history and lifestyle practices (9). A 61-item food frequency questionnaire was added to the Nurses' Health Study questionnaire to obtain dietary information in 1980 and was expanded to 116 items in 1984 and again to 136 items in 1986. In both cohorts, follow-up questionnaires have been mailed to participants every 2 years to update information on potential risk factors for chronic diseases and to ascertain whether major medical events have occurred, and dietary information has been updated every 2–4 years. A question on lifetime occurrence of Parkinson's disease was first included in the 1988 (Health Professionals Follow-up Study) and the 1994 (Nurses' Health Study) questionnaires and subsequently updated every 2 years. Participants who reported Parkinson's disease ( $n = 178$ ), stroke ( $n = 505$ ), or cancer (other than nonmelanoma skin cancer,  $n = 5,572$ ) at baseline were excluded from the analyses. In addition, we excluded from analyses participants with extreme daily energy intakes ( $<800$  or  $>4,200$  kcal for men;  $<500$  or  $>3,500$  for women) or incomplete food frequency questionnaire at baseline ( $>70$  blanks for men or  $>10$  for women). We followed 47,341 eligible men and 88,716 women from baseline (1986 and 1980, respectively) to the date that the first Parkinson's disease symptoms were noticed, the date of stroke diagnosis or death, or the end of the follow-up (January 31, 2000, in men and May 31, 1998, in women), whichever occurred first. These studies were approved by the human subjects committees at the Harvard School of Public Health (Health Professionals Follow-up Study) and Brigham and Women's Hospital (Nurses' Health Study).

#### Parkinson's disease case ascertainment

Ascertainment of the Parkinson's disease cases in this study has been previously described (10). Briefly, after obtaining permission from participants who reported a new diagnosis of Parkinson's disease, we asked the treating neurologist (or internist if the neurologist did not respond) to complete a questionnaire to provide his/her judgment on the certainty of the diagnosis or to send a copy of the medical record. On the questionnaire, we also elicited the information on the date that the first symptoms of Parkinson's disease were noticed and the date when the disease was first clinically diagnosed. A case was confirmed if a diagnosis of Parkinson's disease was considered definite or probable by the treating neurologist or internist, or if the medical record included either a final diagnosis of Parkinson's disease made by a neurologist or evidence at a neurologic examination of at least two of the three cardinal signs (rest tremor, rigidity, bradykinesia) in the absence of features suggesting other diagnoses. The review of medical records was conducted by the investigators who were blind to the exposure status. Overall, the diagnosis was confirmed by the treating neurologist in 82.3 percent of the cases, by review of the medical records in 3.1 percent of the cases, and by the treating inter-

nist without further support in the remaining 14.6 percent of the cases.

#### Exposure assessment

In both cohorts, participants were asked how often, on average, they had consumed a specified amount of each food item in the food frequency questionnaire during the previous 12 months, with nine possible response categories ranging from "never" to "6 or more times per day." Information on the dose and duration of supplemental use of specific vitamins and multivitamins was collected at baseline in both cohorts and updated in the biennial surveys. The nutrient composition of foods was estimated from the Harvard University Food Composition Database that was derived from the US Department of Agriculture (11) and supplemented with information from manufacturers (8) and data from peer-reviewed literature. Validations studies have revealed high correlation coefficients between nutrient intakes estimated from the food frequency questionnaires and those from weighed dietary records. The coefficients were 0.77 for folate, 0.85 for vitamin B<sub>6</sub>, and 0.56 for vitamin B<sub>12</sub> in the Health Professionals Follow-up Study (8) and 0.58 for vitamin B<sub>6</sub> in the Nurses' Health Study (12). Moreover, the correlation between total folate intake and erythrocyte folate concentration was 0.55 in a sample of 188 Nurses' Health Study participants (13). In the control group of a recent Nurses' Health Study on breast cancer (14), intake of these vitamins was also moderately correlated with their plasma concentrations, the coefficients being 0.49 for folate, 0.52 for vitamin B<sub>6</sub>, and 0.25 for vitamin B<sub>12</sub>.

#### Statistical analyses

In the primary analyses, baseline nutrient intake was classified into quintiles, adjusting for energy intake with the residual method (15). Multivariate-adjusted relative risks were derived from Cox proportional hazard models controlling for age (years), smoking status (never smoker, past smoker, or current smoker: cigarettes/day, 1–14 or  $\geq 15$ ), total energy intake (quintiles), caffeine intake (quintiles), alcohol consumption (g/day, men: 0, 1–9.9, 10–19.9, 20–29.9, or  $\geq 30$ ; women: 0, 1–4.9, 5–9.9, 10–14.9, or  $\geq 15$ ), and lactose intake (quintiles). The  $p$  value for linear trend was calculated by using the median of each quintile category as a continuous variable in the Cox models. Log relative risks from the two cohorts were pooled by the inverse of their variances. For all relative risks, we calculated 95 percent confidence intervals and two-tailed  $p$  values. To examine the possibility that folate affects the risk of Parkinson's disease at only high or low levels, we further categorized folate intake into 10 categories, ranging from 200 or less  $\mu\text{g}$  to more than 1,000  $\mu\text{g}$  per day. In a secondary analysis, we also compared participants with intakes of all three vitamins in the top tertile with those with intakes all in the bottom tertile. To take advantage of the repeatedly collected dietary information, we also conducted cumulative updated analyses by relating the average nutrient intake from all surveys prior to the beginning of each biennial questionnaire to the risk of Parkinson's disease in the following 2-year period (16). As

**TABLE 1. Age-adjusted population characteristics according to baseline total folate intake quintile in the Health Professionals Follow-up Study (1986) and the Nurses' Health Study (1980)\***

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5
<b>Men</b>					
Median of intake ( $\mu\text{g/day}$ )	244	317	388	517	841
Current smokers (%)	15.6	10.8	7.6	7.8	8.5
Past smokers (%)	43.2	43.1	43.8	42.8	44.5
Caffeine intake (mg/day)	301	252	221	211	212
Alcohol intake (g/day)	13.0	11.9	10.7	10.6	10.6
Lactose intake (g/day)	14.0	15.0	15.5	15.5	15.4
Vitamin B <sub>6</sub> intake (mg/day)	3.6	4.3	5.6	7.9	22.1
Vitamin B <sub>12</sub> intake ( $\mu\text{g/day}$ )	8.6	9.4	10.4	13.1	21.9
<b>Women</b>					
Median of intake ( $\mu\text{g/day}$ )	158	217	277	393	699
Current smokers (%)	37.1	30.1	26.8	25.2	25.0
Past smokers (%)	23.4	26.6	28.3	29.5	30.6
Caffeine intake (mg/day)	437	410	394	376	368
Alcohol intake (g/day)	6.6	6.6	6.3	6.2	6.2
Lactose intake (g/day)	10.8	13.1	14.3	14.8	15.5
Vitamin B <sub>6</sub> intake (mg/day)	1.6	1.9	2.3	3.3	5.8
Vitamin B <sub>12</sub> intake ( $\mu\text{g/day}$ )	5.5	6.1	6.8	9.2	17.1

\* Means and proportions were directly standardized to the age distribution of each cohort.

Parkinson's disease may develop for many years before it can be clinically diagnosed, we also conducted 6-year lag analyses by excluding the first 6 years of follow-up in order to minimize the effect of dietary changes associated with undiagnosed Parkinson's disease on the analyses.

Since lactose intake was associated with an increased risk of Parkinson's disease in the Health Professionals Follow-up Study cohort (17), we repeated our primary analyses on folate and Parkinson's disease separately in individuals with high (quintiles 4 and 5) or low (quintiles 1–3) lactose intake. Further, as alcohol in many ways affects the absorption and metabolism of folate, we stratified folate analyses by baseline alcohol intake (<15 g and  $\geq$ 15 g) in both men and women. Finally, stratified analyses were also conducted for all nutrients according to baseline age (<65 years vs.  $\geq$ 65 years in men; <50 vs.  $\geq$ 50 years in women) or smoking status (never smokers vs. ever smokers). The cutoff points for age group were selected on the basis of sample size considerations.

## RESULTS

During an average of 12.7 years of follow-up in men and 17.3 years in women, we identified a total of 248 male and 167 female Parkinson's disease patients. The person-year contribution by each 5-year age group during the follow-up was 108,659 (<age 50 years), 103,624 (ages 50–54 years), 96,994 (ages 55–59 years), 92,607 (ages 60–64 years), 84,294 (ages 65–69 years), 64,388 (ages 70–74 years), 36,589 (ages 75–79 years), and 14,522 (age  $\geq$ 80 years), respectively, in men, and 244,414 (ages <45 years), 254,099

(ages 45–49 years), 310,993 (ages 50–54 years), 306,213 (ages 55–59 years), 229,013 (ages 60–64 years), 137,721 (ages 65–69 years), and 54,614 (age  $\geq$ 70 years), respectively, in women. The average baseline intake of folate was 482  $\mu\text{g/day}$  for men and 366  $\mu\text{g/day}$  for women. Among both men and women, participants with higher folate intake were less likely to be current smokers, had lower coffee consumption, and had higher intakes of vitamins B<sub>6</sub> and B<sub>12</sub> (table 1).

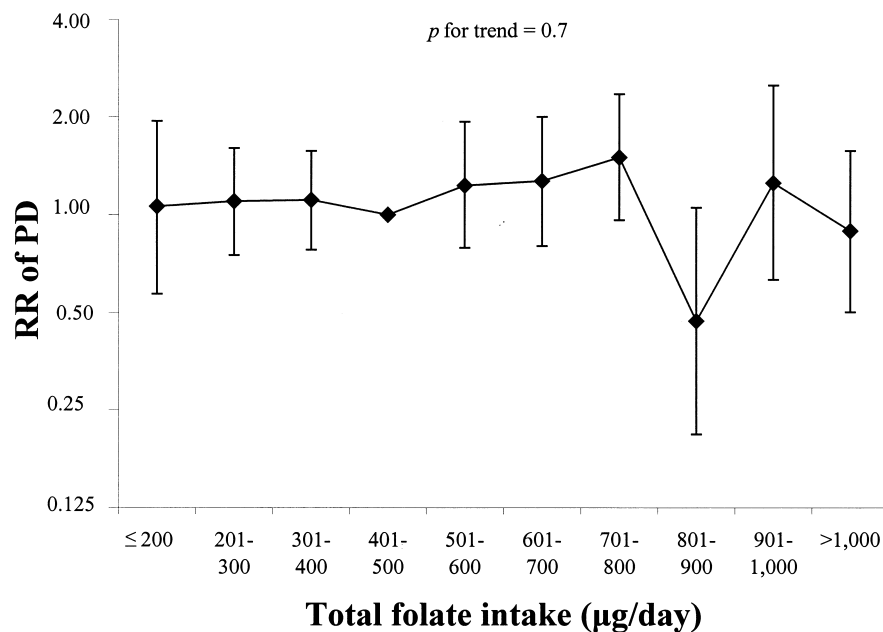
Baseline folate intake was not associated with the risk of Parkinson's disease (table 2). The pooled relative risks comparing the highest and the lowest intake quintiles were 1.1 (95 percent confidence interval (CI): 0.8, 1.5;  $p_{\text{trend}} = 0.8$ ) for total folate and 1.2 (95 percent CI: 0.8, 1.7) for dietary folate. Additional analyses that restricted Parkinson's disease cases to definite cases or neurologist-diagnosed cases generated similar results. Individuals at either the low end or the high end of folate intake in our study population had a Parkinson's disease risk similar to the risk of those with normal folate intake (figure 1): Using folate intake of 400–500  $\mu\text{g/day}$  as the reference group, we found that the pooled relative risks were 1.1 (95 percent CI: 0.6, 1.9) for less than 200  $\mu\text{g/day}$  and 0.9 (95 percent CI: 0.5, 1.6) for more than 1,000  $\mu\text{g/day}$ . The pooled relative risks associated with cumulatively updated folate intake quintiles were 1.0 (referent), 0.9, 1.4, 1.3, and 1.0 for quintiles 1–5, and the corresponding relative risks in the 6-year lag analyses were 1.0, 0.9, 1.2, 1.0, and 1.1, respectively. As with folate intake, no significant association was found between intake of vitamin B<sub>6</sub> or vitamin B<sub>12</sub> and the risk of Parkinson's disease in our analysis. Although a slightly lower risk was found

**TABLE 2. Relative risk\* of Parkinson's disease according to baseline intake of folate, vitamin B<sub>6</sub>, or vitamin B<sub>12</sub> in the Health Professionals Follow-up Study (1986–2000) and the Nurses' Health Study (1980–1998)**

	Intake quintiles					<i>P</i> <sub>trend</sub>
	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	
<b>Total folate</b>						
<b>Men</b>						
No. of cases	39	40	58	53	58	
RR† (95% CI)†	1.0 (referent)	0.8 (0.5, 1.3)	1.1 (0.7, 1.6)	1.0 (0.6, 1.5)	1.0 (0.7, 1.5)	0.9
<b>Women</b>						
No. of cases	21	34	43	31	38	
RR (95% CI)	1.0 (referent)	1.4 (0.8, 2.3)	1.5 (0.9, 2.6)	1.1 (0.6, 1.9)	1.3 (0.8, 2.3)	0.8
Pooled	1.0 (referent)	1.0 (0.7, 1.4)	1.2 (0.9, 1.7)	1.0 (0.7, 1.4)	1.1 (0.8, 1.5)	0.8
<b>Dietary folate</b>						
<b>Men</b>						
No. of cases	32	48	58	55	55	
RR (95% CI)	1.0 (referent)	1.2 (0.8, 1.9)	1.4 (0.9, 2.1)	1.2 (0.8, 1.8)	1.1 (0.7, 1.7)	0.9
<b>Women</b>						
No. of cases	19	28	39	41	40	
RR (95% CI)	1.0 (referent)	1.3 (0.7, 2.3)	1.6 (0.9, 2.7)	1.5 (0.9, 2.6)	1.4 (0.8, 2.4)	0.4
Pooled	1.0 (referent)	1.2 (0.9, 1.8)	1.4 (1.0, 2.0)	1.3 (0.9, 1.8)	1.2 (0.8, 1.7)	0.7
<b>Total vitamin B<sub>6</sub></b>						
<b>Men</b>						
No. of cases	32	52	51	60	53	
RR (95% CI)	1.0 (referent)	1.3 (0.8, 2.0)	1.0 (0.6, 1.6)	1.2 (0.8, 1.8)	1.1 (0.7, 1.7)	0.8
<b>Women</b>						
No. of cases	26	34	34	39	34	
RR (95% CI)	1.0 (referent)	1.1 (0.7, 1.8)	0.9 (0.5, 1.5)	1.1 (0.6, 1.8)	0.9 (0.5, 1.5)	0.7
Pooled	1.0 (referent)	1.2 (0.9, 1.7)	1.0 (0.7, 1.4)	1.1 (0.8, 1.6)	1.0 (0.7, 1.4)	0.7
<b>Dietary vitamin B<sub>6</sub></b>						
<b>Men</b>						
No. of cases	32	50	59	43	64	
RR (95% CI)	1.0 (referent)	1.3 (0.8, 2.0)	1.3 (0.9, 2.0)	0.8 (0.5, 1.3)	1.0 (0.6, 1.6)	0.3
<b>Women</b>						
No. of cases	23	24	40	38	42	
RR (95% CI)	1.0 (referent)	0.9 (0.5, 1.6)	1.4 (0.8, 2.3)	1.1 (0.7, 1.9)	1.1 (0.6, 1.8)	0.7
Pooled	1.0 (referent)	1.1 (0.8, 1.6)	1.3 (1.0, 1.9)	0.9 (0.7, 1.3)	1.0 (0.7, 1.4)	0.5
<b>Total vitamin B<sub>12</sub></b>						
<b>Men</b>						
No. of cases	45	45	57	50	51	
RR (95% CI)	1.0 (referent)	0.9 (0.6, 1.4)	1.1 (0.8, 1.7)	0.9 (0.6, 1.3)	0.9 (0.6, 1.4)	0.6
<b>Women</b>						
No. of cases	41	21	34	43	28	
RR (95% CI)	1.0 (referent)	0.7 (0.4, 1.2)	1.0 (0.6, 1.6)	1.0 (0.7, 1.6)	0.6 (0.4, 1.0)	0.2
Pooled	1.0 (referent)	0.9 (0.6, 1.2)	1.1 (0.8, 1.5)	0.9 (0.7, 1.3)	0.8 (0.6, 1.1)	0.2
<b>Dietary vitamin B<sub>12</sub></b>						
<b>Men</b>						
No. of cases	48	52	40	50	58	
RR (95% CI)	1.0 (referent)	1.1 (0.7, 1.6)	0.8 (0.5, 1.2)	0.9 (0.6, 1.3)	1.0 (0.7, 1.4)	0.99
<b>Women</b>						
No. of cases	23	38	41	31	34	
RR (95% CI)	1.0 (referent)	1.5 (0.9, 2.5)	1.5 (0.9, 2.4)	1.0 (0.6, 1.7)	1.0 (0.6, 1.7)	0.4
Pooled	1.0 (referent)	1.2 (0.9, 1.6)	1.0 (0.7, 1.4)	0.9 (0.7, 1.3)	1.0 (0.7, 1.4)	0.7

\* Adjusting for age, smoking, total energy intake, alcohol consumption, caffeine intake, and lactose intake.

† RR, relative risk; CI, confidence interval.



**FIGURE 1.** Multivariate relative risk and 95% confidence intervals of Parkinson's disease according to total folate intake in the Health Professionals Follow-up Study (1986–2000) and the Nurses' Health Study (1980–1998), adjusting for age, smoking, alcohol consumption, caffeine intake, and lactose intake. RR, relative risk; PD, Parkinson's disease.

when a comparison was made of individuals whose intakes of all three vitamins were in the highest tertile and individuals whose intakes were in the lowest (relative risk = 0.8, 95 percent CI: 0.6, 1.3), the association was not statistically significant. Neither did we find a significant association between dietary intake of these vitamins and Parkinson's disease risk in the analyses stratified by age, smoking status, alcohol consumption (folate only), or lactose intake (folate only). Supplemental intake of these nutrients was also not related to the risk of Parkinson's disease (table 3). Compared with nonusers, individuals whose supplemental folate intake was more than 400 µg/day had a pooled relative risk of 1.0 (95 percent CI: 0.8, 1.2).

## DISCUSSION

In this large prospective analysis, we found no association between intake of folate, vitamin B<sub>6</sub>, or vitamin B<sub>12</sub> and the risk of Parkinson's disease. Similar null results were found when we examined folate intake at very low or high levels or examined it together with intakes of vitamin B<sub>6</sub> and vitamin B<sub>12</sub>.

Both the Health Professionals Follow-up Study cohort and the Nurses' Health Study cohort are large prospective cohorts with validated dietary assessments and long follow-ups. Although participants of this study are health professionals who, on average, reported adequate folate intakes, the large size of these cohorts allowed us to explore the effects of folate over a wide range of intake that encompasses the consumption of the majority of the US population (18). Approximately 53 percent of men and 71 percent of

women reported folate intakes lower than 400 µg/day, thus falling into the range in which the plasma homocysteine concentration increases as the folate intake decreases (1). Further, the folate intake assessed in our cohort was associated with lower risk of colon cancer, breast cancer, and coronary heart diseases (19–21).

Previous epidemiologic studies have demonstrated an association between hyperhomocysteinemia and risk of Alzheimer's disease (3). In the Framingham Cohort Study (3), individuals with plasma homocysteine greater than 14.0 µmol per liter had 90 percent higher risk of Alzheimer's disease compared with those with normal concentrations. Elevated homocysteine may increase Alzheimer's disease risk through its deleterious role in endothelial vascular pathogenesis as well as its direct neurotoxic effects (22–27). It potentiates the neurotoxicity of β-amyloid, enhances glutamate excitotoxicity, overstimulates *N*-methyl-D-aspartate receptors, and induces calcium influx into the neurons (25–27). Further, a high homocysteine concentration as well as folate deficiency may decrease glutathione peroxidase activity and reduce tissue concentrations of antioxidant vitamins (28, 29), making neurons more vulnerable to oxidative attacks. Homocysteine may also induce neuron apoptosis by damaging neuron DNA and subsequently depleting neural energy reserves to repair the damages (27, 30). Although these mechanisms have been proposed to explain the association between folate and Alzheimer's disease, most of them could also apply to other neurodegenerative diseases, including Parkinson's disease. In addition to folate, vitamin B<sub>6</sub> and vitamin B<sub>12</sub> also are important cofactors in the one-carbon metabolism, and individuals with low plasma

**TABLE 3. Relative risk\* of Parkinson's disease according to supplemental intake of folate, vitamin B<sub>6</sub>, or vitamin B<sub>12</sub> in the Health Professionals Follow-up Study (1986–2000) and the Nurses' Health Study (1980–1998)**

	Supplement use			<i>P</i> <sub>trend</sub>
	Nonusers	<400 µg/day	≥400 µg/day	
<b>Folate supplement</b>				
<b>Men</b>				
Cases/person-years	155/398,380	39/86,683	54/116,613	
RR† (95% CI)†	1.0 (referent)	1.1 (0.8, 1.6)	1.0 (0.7, 1.4)	0.98
<b>Women</b>				
Cases/person-years	128/1,128,561	8/131,417	31/277,089	
RR (95% CI)	1.0 (referent)	0.6 (0.3, 1.2)	0.9 (0.6, 1.3)	0.4
Pooled	1.0 (referent)	1.0 (0.7, 1.3)	1.0 (0.8, 1.2)	0.6
	Supplement use			
	Nonusers	<1.7 mg/day	≥1.7 mg/day	
<b>Vitamin B<sub>6</sub> supplement</b>				
<b>Men</b>				
Cases/person-years	73/182,383	88/226,073	87/193,221	
RR (95% CI)	1.0 (referent)	0.9 (0.7, 1.3)	1.0 (0.7, 1.3)	0.98
<b>Women</b>				
Cases/person-years	117/1,021,118	7/122,001	43/393,948	
RR (95% CI)	1.0 (referent)	0.5 (0.2, 1.1)	0.9 (0.6, 1.2)	0.4
Pooled	1.0 (referent)	0.9 (0.7, 1.2)	0.9 (0.7, 1.2)	0.7
	Supplement use			
	Nonusers	<2.4 µg/day	≥2.4 µg/day	
<b>Vitamin B<sub>12</sub> supplement</b>				
<b>Men</b>				
Cases/person-years	68/184,524	91/231,080	89/186,073	
RR (95% CI)	1.0 (referent)	1.0 (0.8, 1.4)	1.1 (0.8, 1.5)	0.4
<b>Women</b>				
Cases/person-years	63/565,233	58/511,077	46/460,757	
RR (95% CI)	1.0 (referent)	1.0 (0.7, 1.4)	0.8 (0.6, 1.2)	0.3
Pooled	1.0 (referent)	1.0 (0.8, 1.3)	1.0 (0.8, 1.3)	0.96

\* Adjusting for age, smoking, total energy intake, alcohol consumption, caffeine intake, lactose intake, and dietary intake of folate, vitamin B<sub>6</sub>, or vitamin B<sub>12</sub>.

† RR, relative risk; CI, confidence interval.

concentrations of these B vitamins are more likely to have hyperhomocysteinemia (1). Although they have not been previously investigated in relation to dopaminergic neuron survival or to the risk of Parkinson's disease, low intakes of these two vitamins, particularly of vitamin B<sub>12</sub>, are probably associated with cognitive declines and the risk of Alzheimer's disease in elder populations (31–33).

Interestingly, individuals with Parkinson's disease have had higher plasma homocysteine concentrations than those without the disease (4–6). This increase may reflect dietary changes after Parkinson's disease diagnosis or may be related to the long-term use of levodopa in Parkinson's disease patients, which may deplete the intracellular methyl group, increase homocysteine concentrations, and promote

its extracellular export (6, 34). However, the higher concentration of plasma homocysteine is also consistent with the possibility that homocysteine itself is neurotoxic to dopaminergic neurons and thus increases the risk of Parkinson's disease. In an experimental study (7), folate deficiency resulted in a high plasma homocysteine level in mice and significantly sensitized dopaminergic neurons to the neurotoxicity of a subtoxic dose of MPTP. This dose did not induce dopaminergic neuron death in mice with adequate folate intake, but it caused a significant decrease in the number of dopaminergic neurons and induced profound motor dysfunctions when combined with a folate-deficient diet. Moreover, focal administration of homocysteine into either striatum or substantia nigra also exacerbated the

MPTP-induced motor dysfunctions and loss of striatal dopamine and its metabolites (7). Further, *in vitro* administration of homocysteine significantly enhanced the neurotoxicity of rotenone or ferrous iron to human dopaminergic neurons (7).

Some limitations should be considered in the interpretation of our findings. In both cohorts, we relied on the clinical diagnosis of the treating neurologist, which in a recent clinicopathologic investigation was found to be accurate in 90 percent of the cases (35). Thus, although the bias from diagnostic misclassification cannot be excluded, it is likely to be modest. Our previous reports on the well-known inverse associations between smoking and coffee consumption (in men only) and Parkinson's disease risk provide further indirect evidence against a substantial diagnostic inaccuracy in the study (10, 36). In studies on diet and chronic diseases, errors in dietary assessment are inevitable (37). We have tried to minimize the measurement error by using validated dietary data and by repeating analyses with cumulatively updated nutrient intake. Further, dietary intakes of folate and related B vitamins are only moderately correlated with plasma homocysteine concentration (1, 14). Therefore, although the results from our study suggest that folate intake is unlikely to be a major determinant of Parkinson's disease risk, they do not exclude the possibility of a mild to modest association between hyperhomocysteinemia and the risk of Parkinson's disease. It is advisable that future prospective studies examine directly the association of Parkinson's disease risk with plasma concentrations of homocysteine, folate, vitamin B<sub>6</sub>, or vitamin B<sub>12</sub>. Further, interactions between folate status and genetic polymorphisms of methylenetetrahydrofolate reductase should also be considered as individuals with these polymorphisms are more likely to have hyperhomocysteinemia, particularly when combined with low folate status (38). Because the participants of this study were health professionals, they were more likely to have adequate folate intake and less likely to have occupational or environmental exposures to neurotoxins, such as pesticides and heavy metals that have been related to Parkinson's disease risk (39, 40). Therefore, we were unable to detect any detrimental effects of very low folate intake or preventive effects of folate in the presence of occupational or environmental neurotoxins.

In summary, the results of this large prospective study among US health professionals suggest that dietary intake of folate, vitamin B<sub>6</sub>, or vitamin B<sub>12</sub> is not related to risk of Parkinson's disease. If high intake of folate reduces the risk of Parkinson's disease, its beneficial effect is most likely restricted to individuals who are exposed to neurotoxins or who are genetically at risk of hyperhomocysteinemia.

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