Prodromal Parkinson's Disease and its Implications for Longitudinal Studies

HARVARD UNIVERSITY

Graduate School of Arts and Sciences

DISSERTATION ACCEPTANCE CERTIFICATE

The undersigned, appointed by the
Committee on Higher Degrees in Population Health Sciences,
have examined a dissertation entitled

"Prodromal Parkinson's Disease and Its Implications for Longitudinal Studies"

presented by

MARIO H. FLORES-TORRES

candidate for the degree of Doctor of Philosophy
and hereby certify that it is worthy of acceptance.

Alberto Ascherio

Dr. Alberto Ascherio, M.D., Dr.P.H., Committee Chair, Harvard T.H. Chan School of Public Health

Deborah Blacker
Deborah Blacker (Aug 17, 2022 09:19 PDT)

Dr. Deborah Blacker, M.D., Sc.D., Harvard Medical School, Harvard T.H. Chan School of Public Health

Brian Healy (Aug 17, 2022 12:15 EDT)

Dr. Brian Curran Healy, Ph.D., Harvard Medical School, Harvard T.H. Chan School of Public Health

Michael Schwarzschild

Dr. Michael A. Schwarzschild, M.D., Ph.D., Massachusetts General Hospital

Date: 16 August 2022

Prodromal Parkinson's Disease and its Implications for Longitudinal Studies

A dissertation presented by

Mario H. Flores Torres

to

The Department of Epidemiology

in partial fulfillment of the requirements

for the degree of

Doctor of Philosophy

in subject of

Population Health Sciences (Concentration: Epidemiology)

Harvard University

Cambridge, Massachusetts

August, 2022

Mario H. Flores Torres

Dissertation Advisor: Professor Alberto Ascherio

Prodromal Parkinson's Disease and its Implications for Longitudinal Studies

Abstract

Parkinson's disease (PD) is a neurodegenerative disorder characterized by movement abnormalities such as resting tremor, bradykinesia, and rigidity. In addition, non-motor symptoms such as decreased ability to smell, constipation, and sleep disorders are often present years before the clinical diagnosis of PD, also known as the prodromal period. PD affects close to one million Americans and its prevalence is expected to grow as the population ages. Existing treatments are not known to stop or slow the progression of the disease and few potential preventive factors have been proposed. The limited number of preventive factors may be due to the lack of large prospective studies with enough follow-up to enable the assessment of long-term exposures while accounting for the long prodromal phase of PD.

In Chapter 1, we used data from 804 men participating in the Health Professionals' Follow-up Study (HPFS) to describe how experiencing combinations of hyposmia, constipation, and probable REM sleep behavior disorder (pRBD), common non-motor features of prodromal PD, are related to objective and subjective measures of cognitive function. In line with previous investigations, we found that the objective cognitive performance was worse in individuals with prodromal PD features, particularly among those with all 3 features, who are probably in the prodromal phase of PD. These men were also more likely to experience subjective cognitive decline (SCD) than men with no prodromal features encouraging further investigation of this marker.

In Chapter 2, we used data from 12,427 women from the Nurses' Health Study (NHS), to examine whether SCD is more likely to be present in women with features suggestive of prodromal PD, compared to women without these features. Women experiencing the 3 examined non-motor features (hyposmia, constipation and pRBD) had the worst mean SCD score and the highest odds of poor subjective cognition. This association persisted when women with objective cognitive deficits were excluded from analyses. SCD was also more common in women with a probability of prodromal PD ≥0.80, based on the MDS score, particularly among those younger than 70 years. Our study suggests that SCD is common among individuals who are probably in the prodromal phase of PD. Our SCD assessment may be a time- and cost-efficient tool for large scale screening for prodromal PD, particularly in combination with other prodromal markers and in younger populations.

In chapter 3, we leveraged longitudinal data from the NHS and HPFS collected over ~30 years of follow-up and investigated the association between long-term intake of folate, vitamin B6, and vitamin B12 with the subsequent occurrence of PD. We did not observe an association between intake of folate or vitamin B6 and the risk of PD. Results for vitamin B12 suggested a modest inverse association, particularly for baseline intake. Recent intake of folate, B6 and B12 supplements tended to be associated with higher PD risk, a trend consistent with reverse causality. Our study leaves open the possibility of a protective effect of vitamin B12 on the development of PD and highlights the implications of reverse causality when studying risk or protective factors for PD.

Table of contents

List of Figures

List of Tables

Acknowledgements

I would like to thank my advisor, Dr. Alberto Ascherio, who believed in me since before I came to Harvard and whose advice was always wise. I would like to thank the rest of my Dissertation Advisory Committee, Dr. Blacker, Dr. Healy, and Dr. Schwarzschild, for their thorough feedback on my chapters and their overall support. Thank you to my Mentor in Mexico, Dr. Martin Lajous, who introduced me to the beauty of epidemiology and was key on making my dream of studying at Harvard come true.

Thank you to my mother, Maribel, for her endless love and support and for teaching me not to be afraid of dreaming, because dreams come true when you work hard. Thank you to my brother, Marco, my best friend, who I admire and care for as he does to me. Thank you to my father, Mario, who is always there when I need him. Thank you to the rest of my family and my community, Las Margaritas, Chiapas, Mexico. I feel very fortunate and proud of the community a grew up. A place full of care, love, and laughs.

Thank you to my lovely partner, Isa, whose love and support where vital to keep me afloat during my PhD journey.

And finally thank you to all my friends. Friendship is to me the school of life.

Chapter 1

Cognitive function in men with non-motor features of Parkinson´s disease

Mario H. Flores-Torres, Katherine C. Hughes, Samantha Molsberry, Xiang Gao, Jae H. Kang, Michael A. Schwarzschild, Alberto Ascherio

Abstract

Objective: Subtle cognitive deficits can occur during the prodromal phase of Parkinson's disease (PD), commonly in conjunction with hyposmia. However, little is known about the association between cognitive function and other features suggestive of prodromal PD. We evaluated the association of non-motor prodromal PD features, including hyposmia, constipation, and probable REM sleep behavior disorder (pRBD), with objective measures of cognitive function and self-reported cognitive decline.

Methods: The study population comprised 804 men who responded to a telephone cognitive interview in 2016-17. Participants included 680 individuals with hyposmia, of whom 45 had confirmed PD, and 124 men without hyposmia. Among these men, we evaluated objective cognitive function and subjective cognitive decline to determine whether the presence of non-motor features of prodromal PD was associated with cognitive functioning. Analyses were adjusted for age, physical activity, body-mass index, smoking status, and coffee consumption.

Results: Individuals with non-motor features of prodromal PD had worse objective and subjective cognitive performance relative to men without non-motor features. Cognitive impairment was particularly prevalent among individuals with concurrent hyposmia, pRBD and constipation (multivariate-adjusted odds ratio [OR] = 3.80; 95%CI 1.52, 9.47 for objective poor cognitive function; OR = 8.71; 95%CI 3.18, 23.83 for subjective cognitive decline). As expected, both objective (OR=7.91) and subjective (OR=17.42) cognitive impairment were also more common among men with confirmed PD.

Conclusions: Our study suggests that cognition is commonly affected in individuals with non-motor prodromal PD features, particularly when multiple of these features are present.

Introduction

Subtle cognitive deficits are often present at the time of diagnosis of Parkinson's disease (PD)[1], and affect over 80% of patients 20 years after diagnosis[2]. More recently, it has been reported that, in some individuals, cognitive deficits can be detected during the prodromal phase[3]. However, data on cognitive function in prodromal PD are scarce, and cognitive function in this prodromal stage remains poorly characterized.

Existing studies have been limited by small samples sizes, non-comprehensive assessments of cognitive function, or have assessed cognitive function in isolation or in association with a single feature of prodromal PD. As such, evidence for the value of cognitive function as an individual predictive marker of prodromal PD remains limited. A recent study reported that a higher probability of prodromal PD was associated with lower cognitive performance in a Greek cohort[4]. Although informative, probability scores do not differentiate specific prodromal features, and olfactory loss, a marker of PD and cognitive decline, was not assessed in this study. Further, previous studies have primarily focused on objective measures of cognitive performance and little is known about subjective cognitive performance in the course of PD[5]. Tests of objective measures have limited clinical applicability in the general population and studying subjective cognition might therefore be useful for screening of large populations.

Here, we present the first investigation of objective and subjective cognitive performance among men with and without non-motor features of prodromal PD. More specifically, we describe how experiencing combinations of hyposmia, constipation, and probable REM sleep behavior disorder (pRBD), common non-motor features of prodromal PD, are related to objective and subjective measures of cognitive function.

Methods

Population

This study was conducted in the Health Professionals Follow-up Study (HPFS), a cohort of 51,529 male health professionals (including dentists, pharmacists, optometrists, osteopath physicians, podiatrists, and veterinarians) who were recruited in 1986 and have been actively followed over time for the incidence of numerous health-related conditions, such as cancer, heart disease and PD. In 2012, we started an investigation of prodromal PD (ProPD) among participants in this cohort (HPFS-ProPD). As part of this investigation, all HPFS participants were asked about bowel movement frequency, use of laxatives, and pRBD in 2012. Then, in 2014, a subset of 6,479 men without PD (including 4,172 with either pRBD or constipation, and 2,307 without pRBD or constipation) and 120 PD cases completed an olfactory test and supplementary questionnaire to update constipation and pRBD.

Assessment of non-motor features of Parkinson's Disease

Olfaction was assessed using the Brief Smell Identification Test (B-SIT), a standardized, self-administered forced choice test consisting of a booklet containing 12 odorants; participants are asked to identify each odorant from a list of 4 alternatives[6]. Each participant's olfactory score was calculated by summing the number of correctly identified odors, and hyposmia was defined as an olfactory score in the bottom 10% of individuals who screened negative for pRBD and constipation (score $\leq$7). Constipation was defined as a bowel movement every other day or less frequently, and/or laxative use at least weekly. To assess pRBD, we used an RBD screening question that investigated dream enactment behavior and violent or excessive movement during sleep ("Has your spouse [or sleep partner] told you that you appear to "act out your dreams" while

sleeping [punched or flailed arms in the air, shouted or screamed], which has occurred at least three times?", adapted from the validated Mayo Sleep Questionnaire[7]. This question, but without the specification of dream enactment having occurred at least three times, has been reported to have a high sensitivity specificity (100% and 95%, respectively) for the diagnosis of polysomnography-confirmed RBD in a community-based sample[7].

Telephone cognitive interviews

In 2016-17, a telephone-based cognitive assessment was administered to a subset of the 6,599 HPFS-ProPD participants. All men with hyposmia in the HPFS-ProPD substudy (1,180 men), and a random sample of 197 PD-free men without hyposmia, pRBD, or constipation from the substudy were invited to participate in the cognitive interviews. Because having multiple prodromal features is strongly associated with PD in this cohort[8], the combination of selection into the ProPD substudy, based on constipation and pRBD, and then into the cognitive interview group, based on hyposmia, was designed to purposefully select men with key prodromal features who were most likely to be in the prodromal phase of PD. The telephone-based cognitive assessment was completed by 693 individuals with hyposmia (59% of those eligible), of whom 46 had confirmed PD, and 125 without hyposmia (63% of those eligible). Overall, characteristics were similar between responders and non-responders, but non-responders appeared to be more obese and less physically active (**Table 1.1**). Fourteen responders with a history of stroke were excluded, resulting in a final sample size of 804.

Table 1.1. Age-adjusted characteristics of all participants selected for cognitive testing according to response status.

	Non-responders (n=559)	Responders (n=818)
Age, years[a]	79.6 (6.0)	78.7 (5.8)
Body mass index, kg/m	26.1(3.9)	26.0(3.6)
Body mass index, categories		
- Normal weight (< 25 kg/m2), %	41.5	42.0
- Overweight (25 kg/m2 - < 30 kg/m2), %	42.1	46.8
- Obese (≥30 kg/m^2), %	16.4	11.2
Smoking status		
- Never smoke, %	35.9	40.1
- Ever smoke, %	47.2	45.2
- Unknown, %	16.9	14.7
Physical activity, met-h/week[b]	33.6(37.0)	38.7(35.9)
Coffee, servings/day	1.1(1.2)	1.1(1.3)
Alcohol, g/day	12.0(14.6)	13.0(14.6)
Depressive symptoms, %	13.3	11.7
PD and prodromal feature status		
- None, %	13.0	15.2
- Hyposmia only, %	25.9	27.6
- Hyposmia and constipation, %	27.7	18.9
- Hyposmia and pRBD, %	13.9	17.8
- Hyposmia, constipation, and pRBD, %	13.6	15.0
- Confirmed PD, %	5.8	5.5

Values are means (SD) or percentages and are standardized to the age distribution of the study population. Values of polytomous variables may not sum to 100% due to rounding. [a]Value is not age adjusted. [b]Metabolic equivalents from recreational and leisure-time activities. 24 observation were excluded when estimating frequencies for depressive symptoms due to missing data.

Trained interviewers who were unaware of the study hypothesis and of participants' disease status completed the telephone-based cognitive assessments. The interview included the following cognitive tests: Telephone Interview for Cognitive Status (TICS)[9], delayed recall of the TICS 10-word list, East Boston Memory Test (EBMT, immediate and delayed recall)[10], animal naming test of verbal fluency[11], the digit span backward test[12], and the Oral Trail Making Test (OTMT) A and B[13]. This assessment has been previously used among HPFS participants[14]. A correlation of 0.81 was found when comparing the global score from the telephone-administered interview to the global score from an in-person interview[15]. Further description of these tests is provided in **Supplemental table 1.1**.

A list of 7 yes/no questions was included at the beginning of the interview to assess subjective cognitive decline (SCD)[16]. These 7 items enquire about recent change in memory and a recent change in ability to: remember recent events; remember a short list of items; remember things from one second to the next; understand or follow spoken instructions; follow a group conversation or plot of a television program; and find one's way on familiar streets.

Ascertainment of PD cases

Our procedure for identifying PD cases has been previously described[21]. Briefly, PD cases are initially identified via biennial self-report questionnaires sent to the entire HPFS cohort in which participants are asked to report new disease diagnoses. The self-reports are then validated by a medical record review conducted by a neurologist specializing in movement disorders. Cases are confirmed if the medical record included either a final diagnosis of PD by a neurologist, or evidence of at least 2 of the 3 cardinal signs (rest tremor, rigidity, bradykinesia) in the absence of features suggesting other diagnoses.

Statistical analysis

To evaluate objective cognitive function, scores for each cognitive test were reversed, if needed, so that higher scores would indicate better performance, and then converted to z-scores, defined as the difference between the participant's score on that test and the mean score among all participants, divided by the standard deviation. Our primary outcome was a global score of cognitive function calculated by averaging the z-scores for all tests. An additional measure of global cognition was based on the TICS. Domain scores were also created by averaging z-scores for all tests pertaining to a specific cognitive domain; attention and language were assessed by using a single test (**Supplemental table 1.1**). Due to the nature of our cognitive interview, we were unable to assess visuospatial function. To identify groups with or without relative cognitive impairment, dichotomized variables were calculated based on a score more than 1 standard deviation (SD) below the group standardized mean, within the range recommended to identify mild cognitive impairment in PD[17]. For the TICS, an established cutoff score of less than 31 points was used to define relative cognitive impairment[18].

A SCD score was created by giving 1 point for every "yes" answer and then categorized as "good" (0 points), "moderate" (1-3 points), and "poor" (4-7 points), based on the distribution in the study population.

The following 6 distinct groups were defined for comparison: (1) individuals with no signs of prodromal PD, (2) individuals with hyposmia only, (3) individuals with hyposmia and pRBD (no constipation), (4) individuals with hyposmia and constipation (no pRBD), (5) individuals with hyposmia, pRBD and constipation, and (6) individuals with confirmed PD. Group 1 was used as reference.

In our analyses, we considered the following potential confounding variables: age at time of assessment (years, continuous); physical activity (met-h/week, quartiles); body-mass index (normal weight <25 kg/m^2, overweight 25 kg/m^2-<30 kg/m^2, and obese ≥30 kg/m^2); smoking status (never, ever, unknown); alcohol consumption (g/day, continuous); and coffee consumption (servings/day, continuous). Information on these variables was obtained from the most recent HPFS questionnaire at the time of analysis (2010 and 2012). Depressive symptoms (Mental Health Inventory[24] score in the bottom 10% of the study population; score ≤21), assessed in the 2014 supplementary questionnaire, were considered in models of SCD. Analyses were not adjusted for education as participants were all health professionals with post-graduate educations and 94% of them reported having a doctorate-level degree in the cognitive interview.

For analyses of objective cognitive function, age- and multivariate-adjusted linear regression models were used to estimate mean z-score differences and 95% confidence interval (95% CI) in global and domain-specific scores between the comparison groups. Logistic regression models were used to estimate age- and multivariate-adjusted odd ratios (OR) and 95%CI of cognitive impairment.

For analyses of subjective cognitive performance, age- and multivariate-adjusted Poisson regression models were used to assess the SCD score as a count variable. Overdispersion was accounted for by scaling the deviance parameter. Age- and multivariate-adjusted multinomial logistic regression were used to further estimate the relative odds for moderate and poor subjective cognition versus good subjective cognition.

This study was approved by the Human Research Committee at the Brigham and Women's Hospital and Harvard T. H. Chan School of Public Health. All participants provided

informed consent. Statistical analyses were performed in SAS version 9.4 (SAS Institute, Cary, NC). P-values were considered significant at the alpha<0.05 level.

Results

Age-adjusted characteristics of participants according to PD status are presented in **Table 1.2**. Depressive symptoms were more prevalent in men with co-occurring constipation, pRBD, and hyposmia and in those with confirmed PD; men in these categories were also less physically active.

Table 1.2. Age-adjusted characteristics of the study population (n=804) according to presence of prodromal features and confirmed PD.

	Prodromal features					Confirmed PD
	No features (n=124)	Hyposmia only (n=217)	Hyposmia and constipation (n=150)	Hyposmia and pRBD (n=146)	Hyposmia, constipation, and pRBD (n=122)	(n=45)
Age, years[a]	78.2(5.7)	79.1(5.8)	79.5(6.2)	78.0(5.4)	78.8(5.5)	76.9(5.6)
Body mass index, kg/m	26.5(4.1)	25.7(3.6)	26.1(3.8)	25.5(3.1)	26.3(3.1)	25.6(3.6)
Body mass index, categories						
- Normal weight (< 25 kg/m2), %	40.7	45.1	39.5	46.3	34.8	53.5
- Overweight (25 kg/m2 - < 30 kg/m2), %	46.4	44.3	47.8	46.1	53.2	34.7
- Obese (≥30 kg/m^2), %	12.9	10.6	12.7	7.6	11.9	11.7
Smoking status						
- Never smoke, %	47.3	37.5	38.1	41.0	44.3	35.0
- Ever smoke, %	39.0	43.2	46.8	43.1	47.9	48.2
- Unknown, %	13.7	19.3	15.1	16.0	7.9	16.8
Physical activity, met-h/week[b]	42.3(35.1)	43.0(37.8)	36.5(31.2)	39.0(40.3)	31.1(30.8)	28.4(28.4)
Coffee, servings/day	1.1(1.4)	1.2(1.4)	1.1(1.3)	1.0(1.2)	0.8(1.0)	0.7(1.0)
Alcohol, g/day	11.9(14.5)	12.1(13.5)	12.5(13.7)	14.5(15.9)	12.9(13.3)	15.1(20.5)
Depressive symptoms, %	8.5	10.4	8.4	10.6	17.2	27.1

Values are means(SD) or percentages and are standardized to the age distribution of the study population. Values of polytomous variables may not sum to 100% due to rounding. [a]Value is not age adjusted. [b]Metabolic equivalents from recreational and leisure-time activities. 13 observation were excluded when estimating frequencies for depressive symptoms due to missing data.

Objective cognitive function

Men in all groups defined by the presence of prodromal features had significantly lower global cognitive mean z-scores than those in the reference group in multivariable-adjusted analyses (**Figure 1.1**); mean score differences indicating worse cognitive performance were particularly pronounced among men with co-occurring constipation, pRBD, and hyposmia (z-score difference= -0.46; 95%CI -0.70, -0.23) and those with confirmed PD (z-score difference= -0.81; 95%CI -1.15, -0.47). For the TICS (**Figure 1.1**), mean score differences were statistically significant for participants with hyposmia and constipation, those with 3 features, and those with confirmed PD. When we looked at differences in specific domains (**Figure 1.1**), we found that performance was worse in men with constipation, pRBD, and hyposmia and those with confirmed PD, particularly with respect language/verbal fluency but also for memory and executive function. In contrast, men with only hyposmia performed significantly worse on the memory score exclusively. As expected, individuals with PD had worse cognitive scores for all tests (**Supplemental table 1.1**). The results of analyses modeling the odds of cognitive impairment were consistent with those above. Using men without prodromal features as reference, the OR for cognitive impairment increased with the number of prodromal features and was highest for men with diagnosed PD (**Table 1.3**). In analyses of the individual cognitive domains (**Table 1.3**), having only hyposmia was significantly associated with memory impairment; having hyposmia and either constipation or pRBD but not both was significantly associated with language domain impairment; having hyposmia, constipation, and pRBD was significantly associated with impaired memory, executive function, and language; and having confirmed PD was associated with impaired executive function, and language.

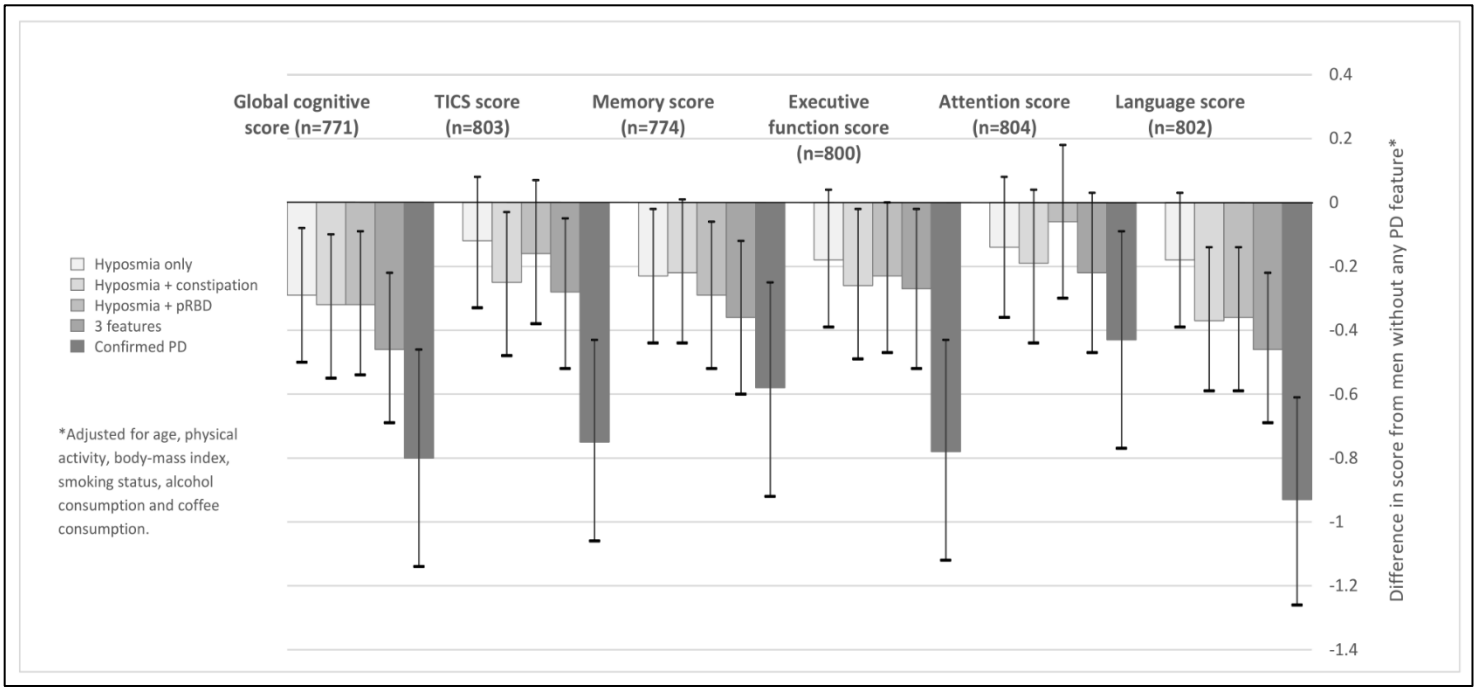


Figure 1.1. Multivariate-adjusted cognitive score differences and 95% CI according to presence of prodromal features and confirmed PD.

Table 1. 3. Adjusted odds ratios of relative cognitive impairment according to presence of prodromal features and confirmed PD.

Measure of cognitive function	No. who completed the test	Prodromal features				Confirmed PD
		No features	Hyposmia only	Hyposmia + constipation	Hyposmia + pRBD	Hyposmia, constipation, and pRBD	
		Odds ratios (95 percent confidence interval)					
Global cognitive score	771						
Age-adjusted		ref	2.89 (1.23, 6.82)	2.85 (1.17, 6.98)	2.74 (1.10, 6.84)	3.92 (1.60, 9.63)	8.58 (2.96, 24.83)
Multivariate-adjusted		ref	2.92 (1.23, 6.94)	2.79 (1.13, 6.89)	2.76 (1.09, 6.95)	3.80 (1.52, 9.47)	7.91 (2.69, 23.27)
TICS score < 31	803						
Age-adjusted		ref	1.20 (0.66, 2.19)	1.86 (1.00, 3.45)	1.44 (0.75, 2.74)	2.19 (1.16, 4.15)	6.26 (2.81, 13.92)
Multivariate-adjusted		ref	1.21 (0.66, 2.22)	1.82 (0.98, 3.40)	1.45 (0.76, 2.79)	2.13 (1.11, 4.09)	6.33 (2.81, 14.23)
Memory score	774						
Age-adjusted		ref	2.37 (1.09, 5.16)	1.30 (0.54, 3.12)	1.82 (0.77, 4.29)	2.98 (1.30, 6.81)	2.06 (0.64, 6.66)
Multivariate-adjusted		ref	2.39 (1.09, 5.25)	1.28 (0.53, 3.10)	1.84 (0.77, 4.41)	3.07 (1.31, 7.18)	2.00 (0.61, 6.59)
Executive function score	800						
Age-adjusted		ref	1.03 (0.51, 2.06)	1.84 (0.91, 3.68)	1.87 (0.93, 3.79)	2.40 (1.18, 4.70)	5.63 (2.44, 12.96)
Multivariate-adjusted		ref	1.01 (0.50, 2.03)	1.83 (0.91, 3.66)	1.86 (0.92, 3.77)	2.42 (1.19, 4.89)	5.74 (2.47, 13.34)
Attention score	804						
Age-adjusted		ref	1.95 (0.53, 7.19)	3.00 (0.82, 11.02)	2.78 (0.73, 10.57)	1.65 (0.38, 7.12)	3.37 (0.64, 17.59)
Multivariate-adjusted		ref	1.87 (0.50, 6.98)	2.65 (0.71, 9.90)	2.39 (0.62, 9.23)	1.49 (0.34, 6.53)	2.45 (0.45, 13.45)
Language score	802						
Age-adjusted		ref	2.23 (0.94, 5.32)	3.23 (1.34, 7.79)	4.18 (1.74, 10.01)	4.29 (1.77, 10.41)	12.04 (4.48, 32.39)
Multivariate-adjusted		ref	2.28 (0.95, 5.47)	3.13 (1.29, 7.60)	4.02 (1.67, 9.71)	3.96 (1.62, 9.71)	11.29 (4.15, 30.70)

TICS denotes the Telephone Interview for Cognitive Status. The global score is a composite of all 8 cognitive tests. The memory score is a composite of 4 tests, the immediate and delayed recalls of the TICS 10-word list and the East Boston Memory Test. The executive function score is a composite of the Digit Span Backwards Test and the Oral Trail Making Test part B. Attention was assessed with the Oral Trail Making Test part A. Language was assessed through the Animal Naming Test for verbal fluency. Multivariate-adjusted odds ratios were adjusted for age (years, continuous), physical activity (met-h/week, quartiles), body-mass index (normal weight, overweight, and obese), smoking status (never, ever, unknown), alcohol consumption (g/day, continuous) and coffee consumption (servings/day, continuous).

Subjective cognitive decline

Of the subjective cognitive complaints we assessed, the most common cognitive concern was having experienced a change in ability to remember things (47.2%) and the least common concern was having experience trouble finding one's way around familiar streets (5.4%). Among study participants, 32.5% reported no cognitive concerns (good subjective cognition), 54.6% reported 1-3 concerns (moderate subjective cognition), and 13.0% reported ≥4 concerns (poor subjective cognition).

In multivariate Poisson regression models, we found that men with only hyposmia, hyposmia and either constipation or pRBD, men with all 3 features, and men with confirmed PD had significantly worse SCD scores compared to those with no PD features (**Figure 1.2**). As in the analyses of objective cognition, the difference in mean SCD scores were greatest in men with co-occurring constipation, pRBD, and hyposmia and in those with confirmed PD, compared to those with no features. Consistent results were obtained in multinomial logistic regression models of subjective cognitive performance (**Table 1.4**). The odds of poor as compared to good subjective cognition were significantly higher for all hyposmic individuals with at least one additional prodromal feature and men with confirmed PD. Exploratory analysis suggested that this association could be driven by the items corresponding to perceived change in ability to remember recent events, follow a group conversation, and find one's way on familiar streets (**Supplemental table 1.3**). Adjusting for depressive symptoms did not change the results in either linear or logistic regression models (**Table 1.4**).

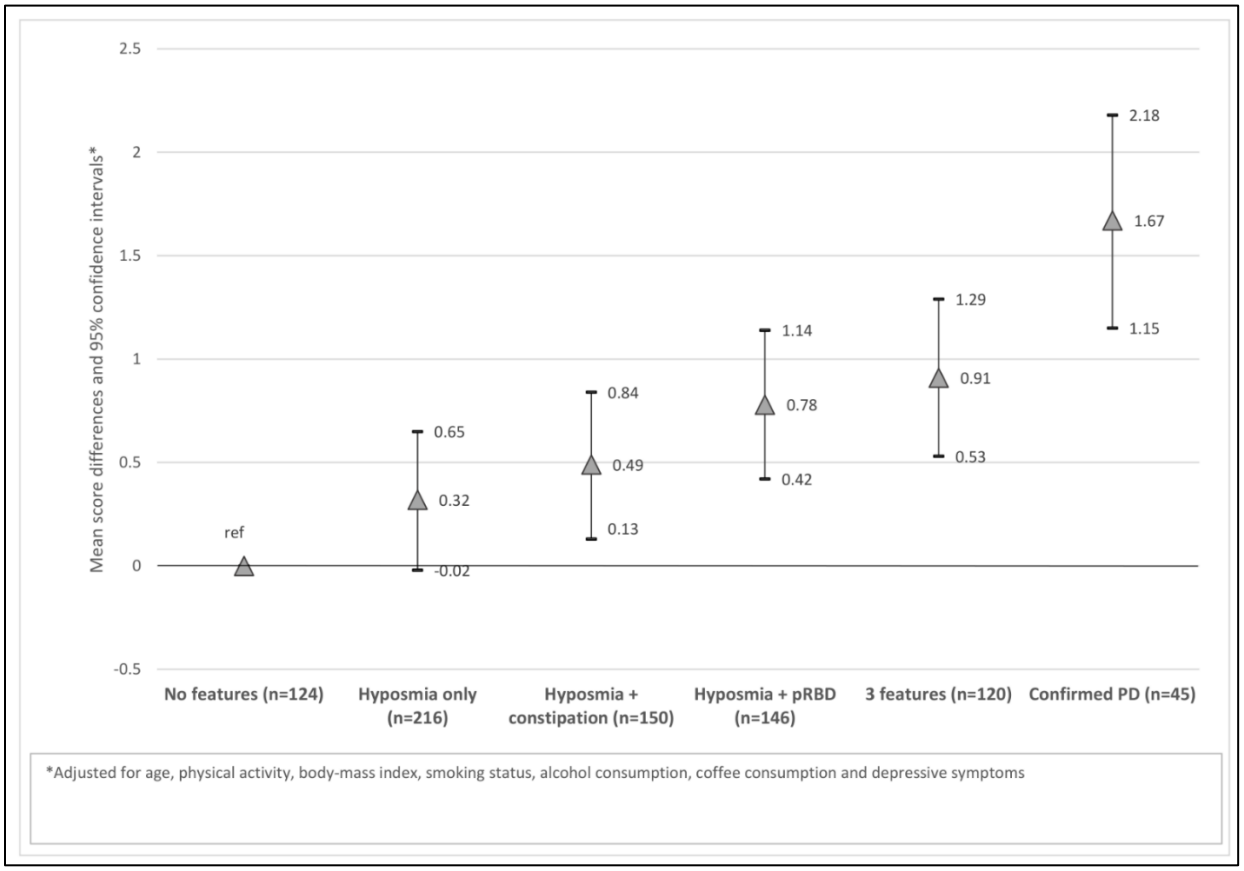


Figure 1.2. Multivariate-adjusted multiplicative increase in the mean subjective cognitive decline score and 95% CI according to presence of prodromal features and confirmed PD (n=801).

Table 1. 4. Adjusted odds ratios of subjective cognition categories according to presence of prodromal features and confirmed PD.

Categories of subjective cognition			**Prodromal features**			**Confirmed PD**
	No features	Hyposmia only	Hyposmia + constipation	Hyposmia + pRBD	Hyposmia, constipation, and pRBD	
			Odds ratios (95 percent confidence interval)			
Good (score 0, reference)						
Number of men	58	80	42	40	30	10
Moderate (score 1-3) versus good						
Number of men	60	119	95	81	63	19
Age-adjusted	ref	1.38 (0.87, 2.20)	2.08 (1.24, 3.50)	2.03 (1.20, 3.45)	2.00 (1.13, 3.54)	2.04 (0.87, 4.81)
Multivariate-adjusted 1	ref	1.44 (0.90, 2.30)	2.20 (1.30, 3.71)	2.05 (1.20, 3.52)	2.02 (1.13, 3.62)	2.05 (0.86, 4.88)
Multivariate-adjusted 2	ref	1.47 (0.92, 2.37)	2.21 (1.30, 3.76)	2.04 (1.19, 3.50)	1.96 (1.09, 3.52)	2.05 (0.86, 4.91)
Poor (score 4-7) versus good						
Number of men	6	17	13	25	27	16
Age-adjusted	ref	1.96 (0.73, 5.29)	2.82 (0.99, 8.06)	6.30 (2.36, 16.84)	8.55 (3.17, 23.07)	17.49 (5.45, 56.05)
Multivariate-adjusted 1	ref	1.99 (0.73, 5.42)	2.93 (1.02, 8.45)	6.29 (2.33, 16.94)	8.71 (3.18, 23.83)	17.42 (5.36, 56.56)
Multivariate-adjusted 2	ref	1.97 (0.72, 5.41)	3.05 (1.05, 8.82)	6.38 (2.36, 17.28)	8.11 (2.95, 22.30)	15.54 (4.70, 51.43)

Multivariate-adjusted 1 included age (years, continuous), physical activity (met-h/week, quartiles), body-mass index (normal weight, overweight, and obese), smoking status (never, ever, unknown), alcohol consumption (g/day, continuous) and coffee consumption (servings/day, continuous). Multivariate-adjusted 2 included all variables from the previous model plus depressive symptoms (yes, no); 16 observations were excluded from the analysis due to missing data on depressive symptoms.

Discussion

In this cross-sectional study of men from the HPFS-ProPD study, we found that individuals with non-motor features suggestive of prodromal PD had worse global cognitive performance than men without these signs. Impairment was particularly pronounced for those with concurrent hyposmia, pRBD, and constipation, who are at higher risk of PD. In addition, hyposmic individuals without other prodromal features performed worse on memory tests, whereas individuals with at least one feature in addition to hyposmia were particularly affected in language/verbal fluency, and, to a lesser extent, in executive function and memory. Hyposmia combined with at least one additional sign was associated with higher odds of poor SCD. Finally, as expected, individuals with confirmed PD performed worse than the rest of the groups in both objective and subjective assessments.

The results of our objective cognitive function analyses provide important insight to further characterize cognitive function in prodromal PD and its relationship with constipation, pRBD, and hyposmia, key non-motor features of prodromal PD. A recent population-based study in Greece found that higher probability of prodromal PD was associated with lower cognitive performance in all cognitive domains, and higher probability of mild cognitive impairment[4]. Another prospective study of 468 participants in Germany showed that future PD converters had lower global cognition scores compared to non-converters years before clinical diagnosis[19]. Similarly, in a case-control study nested in the Rotterdam Study, a subtle decline in executive cognitive functions was found to be present up to 7 years before PD diagnosis[20] and an association between poor cognitive functioning and increased risk of incident parkinsonism, including probable PD, was confirmed in a longitudinal analysis of the same cohort[21]. Our study builds upon these findings by describing the relationship between specific non-motor features of prodromal PD with poor

cognitive function. Similar to our study, results from the PARS study showed that cognitive performance on global cognition, executive function and memory was worse in individuals who were free of PD but had hyposmia and impaired dopamine transporter binding reduction (important predictors of PD)[22]. Results from the same study found that individuals who converted to PD during follow-up, had worse cognitive function at baseline compared to non-converters[23]. Results from the TREND study showed that self-reported forgetfulness and word-finding difficulty were more common in individuals with hyposmia and RBD[24]. Our study expands on these studies by assessing hyposmia, RBD and constipation in the same population and exploring their co-occurrence. In sum, the observations of these studies are consistent with our findings; global cognitive dysfunction or impairment in one of several cognitive domains may be a sign of prodromal PD when occurring in the presence of other relevant non-motor features. Our study addressed some of the limitations of these previous studies by using more extensive measures of cognitive function and robustly assessing some of the most common non-motor prodromal PD features individually and in combination.

Our subjective cognitive performance results are consistent with those based on objective cognitive assessments and with the few studies that have subjectively assessed cognition in prodromal PD. A previous nested case-control study evaluated SCD in prodromal PD and found that PD patients started reporting memory complaints 1.5 years before diagnosis[20]. Another study using a primary care database found that memory problems reported by a clinician were more common in patients with PD compared to those without PD at 2 years before diagnosis[25]. Using a more detailed assessment of current functional abilities, we provide further evidence that SCD might be present in individuals with features suggestive of prodromal PD, particularly in individuals with concurrent hyposmia, constipation, and pRBD. Our results are also in line with

previous studies on cognitive function in PD patients. Robust evidence indicates that, in comparison with age-matched groups without PD, individuals with PD exhibit more rapid decline in many cognitive domains; these are particularly pronounced in the executive, attentional, and visuospatial domains, and, to a lesser extent, memory[5]. In addition, olfactory dysfunction in PD patients has been associated with cognitive impairment[26] and dementia conversion[27]. Because all participants with confirmed PD in our investigation were hyposmic, we could not determine whether hyposmia in PD is associated with more severe cognitive impairment.

Olfactory dysfunction and cognitive impairment are common features not only of prodromal PD but also of early Alzheimer disease (AD)[28] and diffuse Lewy body disease (DLB)[29]; hyposmic individuals in our study might therefore be at higher risk of cognitive decline and it is possible that some of them may develop AD or DLB. The combination of hyposmia, constipation, and pRBD, however, have been strongly associated with PD in this cohort[8], which suggests that men with these features are more likely to be in the prodromal phase of PD rather than AD. In addition, a few studies have suggested a link between cognitive impairment in RBD and the subsequent development of PD or DLB[30, 31]. RBD patients with cognitive impairment are more likely to exhibit non-amnestic cognitive impairment rather than an amnesic phenotype, which seems to be more typical in AD[32]. Therefore, since DLB is notably less common than PD[33], the presence of additional prodromal features such as RBD in hyposmic individuals and the specific nature of their cognitive impairment might help to differentiate those who will potentially develop AD from those who will potentially develop PD.

The evolution and heterogeneity of cognitive impairment in PD mirrors the complexity of the disease process[38 39]. In addition to α-synuclein, tau and amyloid pathologies, many other mechanisms are likely to contribute to cognitive decline, including different neurotransmitter

systems, early synaptic changes, inflammation, and mitochondrial dysfunction[5]. The roles of these and other potentially relevant mechanisms need to be explored further.

Limitations of the current analysis should be considered. First, results reported here are cross-sectional; prospective follow-up of our cohort will be necessary to better characterize the role of cognitive performance in predicting conversion to PD and the development of further cognitive decline, particularly in individuals with additional prodromal features. Second, due to the observational nature of the investigation and use of questionnaires [and/or cognitive test batteries], it is possible that unmeasured or residual confounding and measurement error may be biasing our results. For instance, the performance of individuals with diagnosed PD could have been affected by PD medications, and our assessment of the language domain was based on a single test which may also capture elements of executive function and processing speed. Third, the average age of our cohort at assessment for prodromal features was somewhat older than the average age of onset of PD, and our study was conducted among a homogenous, mostly white male population of health professionals, which could affect the generalizability of the results. Finally, the response rate for the cognitive interviews was relatively low in both hyposmic and non-hyposmic individuals. However, characteristics between responders and non-responders were similar, indicating that non-response likely caused minimal bias in the results.

Strengths of our study are its population-based design and the assessment of multiple prodromal features of PD and their co-occurrence. In addition, potential confounders were robustly assessed, which allowed for careful control of confounding, and well-validated instruments were used to assess both objective and a subjective cognitive performance. The consistency of the results obtained suggests that SCD might have a role in screening of large populations due to its simplicity, low cost, and short application time. Considerable evidence demonstrates that SCD

predicts future cognitive decline in the general population[34], so it might be a harbinger of further cognitive decline in PD.

In conclusion, our study suggests that cognitive impairment is common in individuals with hyposmia, particularly when additional non-motor features of PD, such as constipation and pRBD, are present. The prognostic significance of both subjective and objective measures of cognitive performance and their utility in clinical practice will be determined through longitudinal follow-up currently underway.

Acknowledgments

The authors would like to thank participants of the Health Professionals Follow-up Study for their participation. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

Chapter 2

Subjective cognitive decline in women with features suggestive of prodromal Parkinson's disease

Mario H. Flores-Torres, Kjetil Bjornevik, Albert Y. Hung, Brian C. Healy, Michael A. Schwarzschild, Deborah Blacker, Alberto Ascherio.

Abstract

Background: Cognitive deficits can be present in the prodromal phase of Parkinson's disease (PD). Subjective cognitive decline (SCD) may contribute to identifying individuals with prodromal PD.

Objective: To examine whether SCD is more likely to be present in women with features suggestive of prodromal PD compared to women without these features.

Methods: The study population comprised 12,427 women from the Nurses' Health Study selected to investigate prodromal PD. Prodromal and risk markers of PD were assessed via self-administered questionnaires. We evaluated the association of hyposmia, constipation and probable REM sleep behavior disorder, 3 common features of prodromal PD, with SCD, adjusting for age, education, body mass index, physical activity, smoking, alcohol, coffee consumption, and depressive symptoms. We also explored whether SCD was associated with the probability of prodromal PD and conducted additional analysis using data from neurocognitive tests.

Results: Women experiencing the 3 examined non-motor features had the worst mean SCD score and the highest odds of poor subjective cognition (OR= 1.91; 95% CI: 1.38-2.64). This association persisted when women with objective cognitive deficits were excluded from analyses. SCD was also more common in women with a probability of prodromal PD ≥0.80, particularly among those younger than 70 years (OR of poor subjective cognition = 10.24; 95% CI: 3.07-34.14). These observations were consistent with results from analyses using neurocognitive tests where a worse global cognitive performance was observed among women with 3 features.

Conclusions: Our study suggest that self-perceived cognitive decline can be present during the prodromal phase of PD.

Introduction

Subjective cognitive decline (SCD) is defined as a self-experienced persistent decline in cognitive capacity with a normal performance on standardized cognitive tests[35]. Prospective studies have shown that SCD predicts objective cognitive decline 2-6 years later[34, 36] and it has been proposed as an important early marker of Alzheimer's disease (AD)[37]. In addition, SCD can manifest in the early stages of other disorders in which cognitive function is compromised, and also occurs in those who do not subsequently develop cognitive disorders[38].

Neurocognitive disorders in the form of mild cognitive impairment (MCI) and dementia are common in individuals with Parkinson's disease (PD)[39]. Approximately 10-20% of PD patient present with MCI at the time of diagnosis and close to half develop dementia after 10 years[39]. More recently, it has been reported that objective cognitive deficits can be detected during the pre-diagnostic or prodromal phase of PD[40]. As cognitive deficits become recognized as a feature of prodromal PD[41], SCD may represent a promising marker for large scale screening. Compared to a battery of objective cognitive tests, which can take over an hour to complete and need to be administered by trained personnel, SCD can be assessed in minutes via interview or self-reports. However, little is known about the presence of SCD in prodromal PD.

We aimed to examine whether SCD is more likely to be present in women with features suggestive of prodromal PD compared to women without these features. More specifically, we examined the association between combinations of hyposmia, constipation, and probable REM sleep behavior disorder (pRBD), common non-motor features of prodromal PD, with SCD in a large cohort of US women. To validate our analyses, we evaluated the association between the probability of prodromal PD, as defined by the MDS research criteria[41], and SCD. Finally, we

explored the consistency of our results in a subset population with available data on objective neurocognitive tests.

Methods

Population

The present study was conducted among a subgroup of women from the Nurses' Health Study (NHS) who were purposely selected to investigate prodromal PD: the NHS-ProPD cohort. The NHS is a longitudinal study established in 1976, when 121,700 female nurses responded to a baseline questionnaire about their health and lifestyle. Follow-up questionnaires have been sent biennially to update information on potential risk factors and newly diagnosed diseases. Selection of participants into the NHS-ProPD has been described in detail[42]. Briefly, this cohort started in 2012 and targeted all NHS participants who were <85 years old on January 1st 2012, completed the 2012 follow-questionnaire, had a sleep partner, and were eligible to receive mailings in May 2015 (n=35,480). Based on responses to the 2012 questionnaire, we first assessed the presence of constipation and pRBD. Then, in 2015, all participants with either or both constipation and pRBD, those with diagnosed PD, and a random sample of those without any of these features were mailed a supplementary questionnaire on symptoms of constipation and pRBD and an olfactory test (n=18,838). The olfactory test was returned by 75% (n=14,171) of those eligible; these 14,171 constituted the NHS-ProPD cohort. Our main analyses included the 12,427 NHS-ProPD participants with complete data on covariates (**Supplemental figure 2.1**). Characteristics did not differ between women with and without complete data (**Supplemental table 2.1**).

Subjective Cognitive Decline (SCD)

SCD was assessed in the NHS in 2012 and 2014 as part of the general follow-up via self-report questionnaires. These questionnaires included a list of 7 items inquiring about recent changes in the ability to (i) remember things, (ii) remember recent events, (iii) remember a short list of items, (iv) remember things from one second to the next, (v) understand or follow spoken instructions, (vi) follow a group conversation or plot of a television program and (vii) find one's way on familiar streets[43]. Potential answers were "yes" or "no" and a score was calculated by giving 1 point for every "yes" answer. Among NHS participants, increasing SCD scores have been strongly associated with worse baseline memory and accelerated memory decline[36] as well as with the APOE ε4 allele[44] (an established risk factor for cognitive decline)[45].

Telephone cognitive interviews

Between 2017-2020, a telephone-based cognitive assessment was administered to a subset of 715 NHS-ProPD participants. In addition to the 7 SCD questions, this assessment included a battery of tests to objectively measure cognitive performance (**Supplemental table 2.2)**. These tests have been previously used among NHS participants,[14] and a high correlation (0.81) between telephone and in-person interviews has been reported in this cohort[46]. Although not PD-specific, these types of tests are endorsed by the Movement Disorder's Society for their use in PD research[47]. Due to cost constraints, only a random sample of 40% of all women with hyposmia and 5% of all women without hyposmia, constipation, or pRBD were selected for this interview (n=1,310; **Supplemental figure 2.1**). Among eligible participants, those who responded to this interview were more educated and physically active, had a slightly higher

intake of alcohol and caffeine, and had a lower prevalence of depressive symptoms and poor SCD than those who did not respond (**Supplemental table 2.3**).

Assessment of prodromal markers of Parkinson's Disease

Information to define hyposmia, constipation and pRBD was obtained from the 2015 assessment conducted among NHS-ProPD participants. Olfaction was evaluated using the Brief Smell Identification Test (BSIT),[48] and individuals with a score in the bottom 10% of individuals without pRBD and constipation were considered to have hyposmia. Constipation was defined as self-reported bowel movement every other day or less frequently, and/or laxative use at least weekly[49]. pRBD was assessed using a question adapted from the Mayo Sleep Questionnaire that asks about dream enactment behavior and violent or excessive movement during sleep that has occurred at least three times[7]. The original question was reported to have a sensitivity of 100% and specificity of 95% for the diagnosis of polysomnography confirmed RBD[7]. Using these definitions, we have found strong associations of hyposmia, pRBD, and constipation with PD in the NHS, especially when these features co-occur (OR for PD when all 3 features are present = 211; 95% CI 84, 529; AUC=0.84)[42].

We also collected information on 4 additional prodromal markers: excessive daytime somnolence, urinary dysfunction, depression, and abnormal motor symptoms. Further description of these prodromal makers as well as on risk markers and other covariates of interest is presented in the **Supplemental methods**.

Calculation of the probability of prodromal PD based on MDS research criteria

We calculated the probability of prodromal PD following the updated MDS research criteria[50]. We were able to include information on 7/9 risk makers and 7/9 prodromal markers

applicable to women (**Supplemental table 2.4**). First, the pretest probability was assigned according to MDS estimates as follows: 0.4% from ages 50-54; 0.75% from ages 55-59; 1.25% from ages 60-64; 2.0% from ages 65-69; 2.5% from ages 70-74; 3.5% from ages 75-79; and 4.0% age ≥80. Then, the pretest probability was used to derive the pretest odds (odds=probability/1-probability). The pretest odds were multiplied with the product of the likelihood ratios (LRs) of all risk and prodromal markers (total LR) to yield the posttest odds. MDS estimates of the LRs were assigned as described in **Supplemental table 2.4**. Finally, the posttest probability, that is, the probability of prodromal PD, was derived from the posttest odds (probability=odds/1+odds). We defined a posttest probability ≥80% and ≥50% as probable and possible prodromal PD, respectively.

Ascertainment of PD cases

Procedures for identification of PD cases in the NHS have been previously described[51]. Briefly, PD cases are identified via biennial self-report questionnaires sent to the entire NHS cohort in which participants are asked to report new disease diagnoses and validated by a medical record review conducted by a neurologist specializing in movement disorders. Cases are confirmed if the medical record includes either a final diagnosis of PD by a neurologist, or evidence of at least 2 of the 3 cardinal signs (rest tremor, rigidity, bradykinesia) in the absence of features suggesting other diagnoses.

Statistical analysis

In the primary analyses we used data from 2014-15 to evaluate the cross-sectional association of hyposmia, pRBD, and constipation with SCD. SCD was analyzed as a continuous score and a binary outcome variable indicating poor subjective cognitive function (SCD score ≥

3)[36, 44]. Participants were categorized into having none, 1, 2, or 3 non-motor features based on previous findings in this cohort suggesting that the combination of these features may be useful for identifying populations in the prodromal phase of PD.[42] We explored all possible combinations of these features in secondary analyses. Women with no features were the reference group in all analyses. Women with diagnosed PD served as a positive control in which we expected the degree of cognitive impairment to be the highest. Values from 2012 were carried forward if 2014 data were missing (221 for SCD, 99 for constipation and 3,411 for pRBD).

To further validate our results, we evaluated the association between the probability of prodromal PD and SCD. For this analysis, we excluded all PD cases and assessed how probable, possible and increasing probabilities of prodromal PD (<0.20, 0.20 to <0.40,40 to <0.60, 0.60 to <0.80, and ≥0.80) relate to SCD.

To explore the consistency of our findings, we conducted analyses in a subset of 670 women who responded to the telephone cognitive interview (2017-2020) and had complete data on covariates. Our main outcome was a global score calculated by averaging the z-scores for all tests. A secondary measure of global cognition was based on the TICS. We also created scores evaluating specific cognitive domains (memory, executive function, attention, and language). For both global and domain-specific measures, participants who score below 1 SD of the normative mean (i.e., among those with no features) were classified as having MCI,[47] except for the TICS for which a standard cut-off of <31 is commonly used to define cognitive impairment.[18] SCD was analyzed in a similar way as in the entire study population but using data from the telephone interviews. In addition, to better approximate the formal definition of SCD in which objective impairment is not present, we repeated analyses excluding women with MCI based on the global

cognitive score. Since these interviews were conducted only among hyposmic women (with or without additional features or diagnosed PD) and a random sample of women without non-motor features, the comparison groups for these analyses excluded non-hyposmic women with either constipation or pRBD. Information on PD diagnosis was updated to 2018.

For analyses of SCD, multivariable-adjusted Poisson regression models were used to assess the multiplicative differences in the SCD score and 95% confidence intervals (CI). Multivariable-adjusted logistic regression was used to further estimate the odds ratio (OR) and 95% CI of poor subjective cognition. For analyses of objective cognitive function, multivariable-adjusted linear regression models were used to estimate mean z-score differences and 95% CI in global and domain-specific scores. Logistic regression models were used to estimate adjusted ORs and 95% CI of MCI. For analyses looking at the combination of hyposmia, constipation, and pRBD, models were adjusted for variables that are hypothesized to be associated with both prodromal PD and cognitive function including age, education (associate degree, bachelor's degree, or graduate degree), body mass index (normal weight, overweight, or obese), physical activity (met-h/week), smoking status (never, <5, 5-<25, or ≥25 pack years), alcohol (g/day) and coffee consumption (mg/day), and depressive symptoms (Mental Health Inventory[52] score in bottom 10% of women without pRBD or constipation). Models for the probability of prodromal PD were only adjusted for age and education.

Since age is not only an important component of the prodromal PD score but also a major determinant of cognitive decline, we evaluated the association between prodromal PD and SCD in different age groups (< 70, 70 to 74, 75 to 79, and ≥80 years).

This study was approved by the Human Research Committee at Mass General Brigham health care and Harvard T. H. Chan School of Public Health. All statistical analyses were performed in SAS for UNIX version 9.4 (SAS Institute, Cary, NC). Statistical significance was determined at the 5% level (2-sided).

Results

Characteristics of study participants are presented in **Table 2.1**. Women with co-occurrent hyposmia, constipation, or pRBD were older, less physically active, and more likely to smoke ≥25 pack-years and to report depressive symptoms than women without these features. The prevalence of poor subjective cognition was 11.3%.

Table 2.1. Age-standardized characteristics of the study population according to the presence of prodromal PD features and diagnosed Parkinson's disease (PD).

	Number of features				Diagnosed PD	p-trend[c]
	None	**1**	**2**	**3**		
	(n=3,874)	**(n=6,231)**	**(n=1,941)**	**(n=299)**	**(n=82)**	
Age in years[a]	77.3 (4.9)	77.8 (5.1)	78.8 (5.3)	79.7 (4.8)	79.5 (5.2)	<0.001
Education level, %						
Associate degree	62.8	65.7	64.5	65.2	72.0	0.09
Bachelor's degree	24.1	22.2	23.8	23.7	18.3	0.65
Graduate degree	13.2	12.1	11.7	11.0	9.8	0.06
Phys. Act. meth/w[b]	27.2 (27.7)	23.9 (26.7)	20.6 (24.9)	18.3 (20.7)	15.4 (18.7)	<0.001
Body mass index categories, %						
Normal weight (<25 kg/m^2)	45.4	47.3	44.3	45.5	52.4	0.24
Overweight (25 to <30 kg/m^2)	34.7	33.0	35.3	32.4	24.4	0.55
Obese (≥30 kg/m^2)	19.9	19.6	20.4	22.1	23.2	0.03
Alcohol g/d	7.2 (11.1)	6.6 (10.5)	6.7 (12.6)	6.7 (11.1)	5.5 (12.8)	0.07
Smoking pack-years, %						
Never smoked	49.2	47.9	47.9	40.8	56.1	0.16
<=4 pky	12.8	12.6	12.3	10.4	12.2	0.61
5-24 pky	24.7	24.4	22.9	26.4	29.3	0.55
>=25 pky	13.4	15.1	17.0	22.4	2.4	0.002
Caffeine mg/d	144.1 (131.4)	142.4 (133.8)	138.1 (134)	159.5 (143)	116.4 (135.0)	0.63
Depressive symptoms, %	12.2	16.7	21.7	23.7	34.1	<0.001

Values are means (standard deviations) unless otherwise specified. [a]Value is not age-adjusted. [b]Metabolic equivalents from recreational and leisure-time activities. [c]Values were obtained using age-adjusted linear or logistic regression modeling the exposure as a continuous variable.

Prodromal features and SCD

The presence of hyposmia, constipation, or pRBD was associated with SCD, and more so when 2 or especially all 3 features were present. The multivariable adjusted odds of poor subjective cognition among women with 3 features was 1.91 (95% CI 1.38, 2.64) times that of women without these features (**Table 2.2**). When we looked at specific combination of features, we observed the strongest association for women with hyposmia and pRBD (**Table 2.2**). As expected, the OR of poor subjective cognition was higher in women with PD than in the other groups. Results from Poisson regression models using the SCD score as the outcome were consistent with these findings (**Supplemental figure 2.2**).

Table 2.2. Adjusted odds ratios and 95% confidence interval of poor subjective cognition according to presence of prodromal features and diagnosed Parkinson's disease (PD).

	Cases/non-cases	Age-adjusted	Multivariable
Number of prodromal features			
None	317/3,557	ref	ref
1	699/5,532	1.39 (1.21, 1.60)	1.29 (1.12, 1.48)
2	316/1,625	2.04 (1.73, 2.41)	1.78 (1.50, 2.11)
3	54/245	2.23 (1.62, 3.06)	1.91 (1.38, 2.64)
Diagnosed PD	20/62	3.28 (1.95, 5.52)	2.53 (1.48, 4.32)
Combination of features			
None	317/3,557	ref	ref
Constipation only	498/4,231	1.31 (1.13, 1.51)	1.20 (1.04, 1.40)
pRBD only	86/590	1.73 (1.34, 2.24)	1.60 (1.23, 2.07)
Constipation and pRBD	84/534	1.85 (1.43, 2.40)	1.61 (1.24, 2.10)
Hyposmia only	115/711	1.61 (1.28, 2.02)	1.53 (1.21, 1.94)
Hyposmia and constipation	197/955	2.05 (1.69, 2.48)	1.79 (1.46, 2.18)
Hyposmia and pRBD	35/136	2.72 (1.84, 4.02)	2.32 (1.55, 3.46)
3 features	54/245	2.23 (1.62, 3.06)	1.92 (1.39, 2.65)
Diagnosed PD	20/62	3.29 (1.95, 5.53)	2.53 (1.48, 4.32)

Multivariate models were adjusted for age (years, continuous), education (associate degree, bachelor's degree, or graduate degree), physical activity (met-h/week, continuous), body-mass index (normal weight, overweight, or obese), smoking status (never, <5, 5-<25, or ≥25 pack years), alcohol consumption (g/day, continuous), caffeine intake (mg/day, continuous) and depressive symptoms (yes/no).

Probability of prodromal PD and SCD

Among study participants, 1.9% (235 women) had an estimated probability of prodromal PD ≥80% and 5.6% (700 women) had an estimated probability ≥50%. After adjusting for age and education, the OR for poor subjective cognition comparing women with and without probable prodromal PD was 3.51 (95% CI 2.61, 4.73; **Table 2.3**). An OR of 3.02 (95% CI 2.51, 3.64) was observed when comparing women with and without possible prodromal PD. Similarly, we observed that both the odds of poor subjective cognition (**Table 2.3**) and the mean SCD score (**Supplemental figure 2.3**) increased as the probability of prodromal PD increased. In age-stratified analyses, we observed that the strength of these associations was the highest among women younger than 70 years (**Table 2.3)**.

Table 2.3. Adjusted odds ratios and 95% confidence intervals of poor subjective cognition according to the probability of prodromal PD (PPD).

	Study population[a]	<70y	70-74y	75-79y	≥80y
n	12,345	2,917	4,094	3,319	2,015
Probable PPD (probability ≥80%)					
No	ref	ref	ref	ref	ref
Yes	3.51 (2.61, 4.73)	9.44 (2.83, 31.45)	3.66 (1.86, 7.19)	5.21 (3.24, 8.40)	2.15 (1.30, 3.55)
Possible PPD (probability ≥50%)					
No	ref	ref	ref	ref	ref
Yes	3.02 (2.51, 3.64)	5.95 (3.07, 11.56)	2.39 (1.58, 3.61)	3.60 (2.66, 4.86)	2.66 (1.95, 3.64)
Categories of PPD					
<20%	ref	ref	ref	ref	ref
20% - <40%	2.16 (1.81, 2.57)	1.99 (1.13, 3.50)	2.20 (1.55, 3.11)	2.43 (1.81, 3.26)	1.85 (1.35, 2.55)
40% - <60%	2.50 (1.93, 3.21)	3.82 (1.60, 9.13)	2.66 (1.63, 4.35)	2.39 (1.53, 3.75)	2.20 (1.40, 3.47)
60% - <80%	3.14 (2.39, 4.13)	5.62 (2.39, 13.24)	2.44 (1.29, 4.63)	3.49 (2.22, 5.49)	2.76 (1.73, 4.41)
≥80%	4.35 (3.22, 5.88)	10.24 (3.07, 34.14)	4.14 (2.10, 8.14)	6.53 (4.03, 10.58)	2.68 (1.61, 4.46)

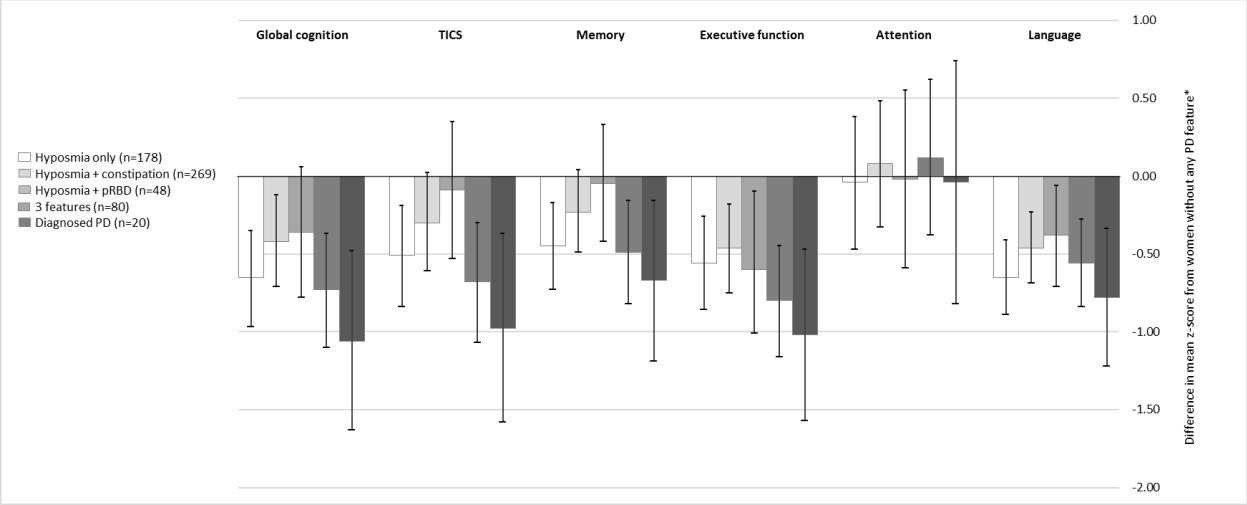
Estimates are adjusted for age (continuous) and education (associate degree, bachelor's degree, or graduate degree). Age-stratified estimates are only adjusted for education. [a]Participants with diagnosed PD were excluded from all analyses.

Subset analysis

When we evaluated the association between prodromal features and the global cognitive score, we observed that that the mean z-scores were lower (indicating worse cognitive performance) in women with prodromal features than in those with no features (**Figure 2.1**). The global cognitive performance was the worst among women with concurrent hyposmia, constipation and pRBD (multivariable z-score difference=−0.74; 95% CI −1.13 to –0.35) and those with PD (multivariable z-score difference=−1.07; 95% CI −1.68 to –0.45). Similarly, women with 3 features and those with PD had the worst performance in most of the cognitive domains evaluated, particularly in executive function, followed by language and memory (**Figure 2.1**). No associations were observed for attention. In addition, women with only hyposmia performed worse than hyposmic women with either constipation or pRBD in most of the analyses. Results from analyses looking at MCI as the outcome of interest were consistent with these findings (**Supplemental table 2.5**).

Among the subgroup of women with available data on objective and subjective measures, estimates from analyses on prodromal features and SCD also suggested a worse SCD among women with 2 and specially 3 features (**Table 2.4; Supplemental figure 2.4).** The association between having all 3 features and SCD was strengthened when women with probable MCI based on objective tests were excluded from the analyses (multivariable adjusted OR=6.60; 95% CI 1.68, 25.93 vs 4.30; 95% CI 1.61, 11.50; **Table 2.4**). This association persisted even when women with only hyposmia, which is also an early marker of AD, were used as the reference (multivariable-adjusted OR=4.16; 95% CI 1.62, 10.67). No substantial change in estimates was observed for the rest of the groups.

Figure 2.1. Adjusted cognitive score differences and 95% confidence intervals according to the presence of prodromal features and diagnosed Parkinson's disease (PD) in the subset population, n=670



*Z-scores were defined as the difference between an individual's score and the mean score among women without hyposmia, pRBD, and constipation, divided by the standard deviation. Mean z-scores differences are adjusted for age (years, continuous), education (associate degree, bachelor's degree, or graduate degree), physical activity (met-h/week, continuous), body-mass index (normal weight, overweight, or obese), smoking status (never, <5, 5-<25, or ≥25 pack years), alcohol consumption (g/day, continuous), caffeine intake (mg/day, continuous) and depressive symptoms (yes/no).

Table 2.4. Adjusted odds ratios and 95% confidence intervals of poor subjective cognition according to presence of prodromal features and diagnosed Parkinson's disease (PD) in the subset population.

	No features	**Hyposmia**				
			+ constipation	+ pRBD	+ constipation and pRBD	Diagnosed PD
All participants, n=670						
Age-adjusted	ref	1.63 (0.63, 4.19)	2.20 (0.90, 5.38)	3.85 (1.34, 11.12)	5.22 (2.00, 13.62)	2.86 (0.72, 11.35)
Multivariable-adjusted	ref	1.34 (0.51, 3.52)	1.71 (0.68, 4.28)	2.88 (0.97, 8.62)	4.30 (1.61, 11.50)	2.26 (0.54, 9.54)
Women without global MCI, n=463						
Age-adjusted	ref	1.91 (0.51, 7.23)	2.28 (0.65, 8.02)	3.50 (0.78, 15.63)	8.18 (2.20, 30.42)	3.00 (0.27, 32.87)
Multivariable-adjusted	ref	1.67 (0.43, 6.56)	1.93 (0.53, 7.01)	2.76 (0.59, 13.01)	6.96 (1.77, 27.37)	1.79 (0.15, 21.78)

Multivariate models were adjusted for age (years, continuous), education (associate degree, bachelor's degree, or graduate degree), physical activity (met-h/week, continuous), body-mass index (normal weight, overweight, or obese), alcohol consumption (g/day, continuous), smoking status (never, <5, 5-<25, or ≥25 pack years), alcohol consumption (g/day, continuous), caffeine intake (mg/day, continuous) and depressive symptoms (yes/no).

Discussion

In this large cross-sectional study of women from the NHS-ProPD study, we found that SCD was more common in individuals with features suggestive of prodromal PD, especially among those experiencing the 3 examined non-motor features. This association persisted even when women with objective cognitive deficits were excluded from analyses. SCD was also more common in women with a higher probability of prodromal PD, based on MDS criteria, particularly among those younger than 70 years. We observed similar results using data from neurocognitive tests where a worse global cognitive performance was observed among women with 3 features. The most affected neurocognitive domains were executive function, followed by language and memory. As expected, the observed associations were stronger among women with confirmed PD than in the rest of the groups. Overall, our study suggests that SCD is present in prodromal PD and could contribute to the early identification of PD cases.

To our knowledge, this is the first study to primarily explore the role of SCD as a potential marker of prodromal PD. Most of the existing studies have focused on objective cognitive deficits[40], and the few studies that have explored the presence of perceived cognitive changes used shorter assessments limited to memory complaints[20, 53-55]. Yet, these studies suggest that perceived memory changes can be present up to 12 years before PD diagnosis which provides support to our findings. In contrast to these studies, our SCD assessment includes questions not only on perceived memory changes but also on changes in executive function (e.g., difficulty following spoken instructions), attention (e.g., trouble remembering things from one second to the next) and visuospatial function (e.g., trouble finding one's way around familiar streets) which are more commonly observed in PD[39]. We were also able to examine the formal definition of SCD in which objective cognitive deficit are not present. Our study thus builds

upon previous efforts and suggests that perceived cognitive changes may be present in prodromal PD, even in the absence of objective cognitive deficits. Our findings are also in line with a similar study we conducted in sample of 804 US men in which the co-occurrence of hyposmia, constipation and pRBD was strongly associated with SCD[56].

An important challenge in our and other studies on prodromal PD is the possibility that some of those who are believed to be in the prodromal phase will never develop PD and may instead develop another disorder. This is particularly important when studying early cognitive changes that are also common in AD[39]. Furthermore, a proposed prodromal symptom of AD is hyposmia[57, 58]. It is thus possible that some of the hyposmic women in our cohort will develop AD instead of PD, which may help explain the worse performance in objective tests observed among women with only hyposmia compared to women with two features. However, it is less likely that hyposmic women with additional features, especially those with all 3 features develop AD. In addition, consistent results were observed when using the MDS probability score which considers additional markers of prodromal PD, and the association between the probability of prodromal PD and SCD was the strongest among those younger than 70 years who are at a lower risk of AD.

In contrast to our finding using objective neurocognitive tests, women with only hyposmia did not appear to be as impaired as women with 2 or 3 features on SCD measures. In addition, when women with MCI based on objective tests were excluded from analyses the OR of poor subjective cognition was strengthened for women with all 3 features, who are probably in prodromal PD. This observation raises the hypothesis that although cognitive deficits captured by objective tests may be present in prodromal PD, its distinction from early AD may be challenging, whereas subtle cognitive changes captured by SCD, which are less AD-specific, can

be better at identifying individuals in prodromal PD, especially when combined with other markers.

Additional limitations should be considered. First, our cross-sectional analyses are limited by the inability to assess temporality. Yet, our main objective is not to establish a temporal relationship, but to describe the presence of SCD in women who are likely in the prodromal PD. Second, the low response rate to the cognitive interview in the subset population could bias our results since non-response may have been differentially determined by the presence of poor subjective cognition (20.7% in non-responders vs 13.0% in responders) and the presence of non-motor features (15.5% in non-responders and 12% in responders). If this is the case, the subset population would be composed of a group of women with prodromal features who are less cognitively impaired than in the source population, which would attenuate the observed associations. Third, some degree of measurement error of prodromal PD features, SCD, and objective cognitive function is inevitable. However, these errors are most likely uncorrelated and are therefore unlikely to explain the hypothesized positive association. Lastly, our study population comprises a relatively homogeneous population of nurses with an average age of 78 years; it is possible that the assessments used in this study would not perform as well in other populations (e.g., women with less education) and that our results may not be transportable to other groups (e.g., populations with a different age distribution).

In conclusion, our study suggests that SCD is common among individuals who are probably in the prodromal phase of PD. Our SCD assessment may be a time- and cost-efficient tool for large scale screening for prodromal PD, particularly in combination with other prodromal markers and in younger populations. The presence of SCD in prodromal PD could also help identify individuals who may later develop MCI and dementia. The predictive value of

SCD needs to be determined in longitudinal studies. Investigations are needed to determine the clinical utility of this measure and its ethical considerations.

Acknowledgment

The authors would like to thank participants of the Nurses' Health Study for their participation.

Chapter 3

Long-term intake of folate, vitamin B6, and vitamin B12 and the incidence of Parkinson's disease in a sample of US women and men.

Mario H. Flores-Torres, Chadwick W. Christine, Kjetil Bjornevik, Samantha A. Molsberry, Albert Y. Hung, Brian C. Healy, Deborah Blacker, Michael A. Schwarzschild, Alberto Ascherio.

Abstract

Background: Folate and vitamins B6 and B12 have been proposed as protective against the development of PD (PD). However, only two longitudinal studies have been conducted, with inconclusive results.

Objective: To examine the association of long-term intake of folate, vitamin B6 and vitamin B12 with the incidence of PD.

Methods: The study population comprised 80,965 women (1984-2016) and 48,837 men (1986-2016) participating in two large US cohort studies. Measures of dietary, supplemental, and total intake of B vitamins were collected at baseline and every 4 years thereafter using a semi-quantitative Food Frequency Questionnaire. Age- and time-stratified Cox regression models were fitted to estimate the hazard ratio (HR) and 95% confidence interval (CI) of PD according to quintiles of cumulative average intake adjusting for potential confounders. Secondary analyses were conducted using different lagged exposure periods as well as baseline and the most recent intakes.

Results: In analyses of *cumulative average* intake, total folate, B6, or B12 were not associated with the risk of PD. Results from 8-, 12-, and 16-year *lag analyses* were consistent with these findings. Results for *baseline* intake of folate and B6 pointed towards a null association with PD. In contrast, a lower PD risk was observed among individuals with higher *baseline* total intake of vitamin B12 (pooled HR top vs bottom quintile: 0.80; 95% CI: 0.67, 0.95; p-trend 0.01); this finding was supported by results from 20-year *lag analyses*.

Conclusions: Our results provide moderate support for a possible protective effect of early vitamin B12 on the development of PD.

Introduction

Parkinson's disease (PD) is a neurodegenerative disorder characterized by movement abnormalities such as resting tremor, bradykinesia, and rigidity.[59] In addition, non-motor symptoms such as decreased ability to smell, constipation, and sleep disorders are often present before the clinical diagnosis of PD,[60] also known as the prodromal period.[41] PD affects close to one million Americans and its prevalence is expected to grow as the population ages.[61] Existing treatments are not known to stop or slow the progression of the disease and few potential preventive factors have been proposed.[59] The limited number of potentially preventive factors may be due to the lack of large prospective studies with enough follow-up to enable the assessment of long-term exposures while accounting for the long prodromal phase of PD.

B vitamins, including folate, vitamin B6, and vitamin B12, have been proposed as protective against the development of PD. In preclinical studies, these vitamins decreased neurotoxic homocysteine levels,[62] facilitated dopamine synthesis,[63] modulated leucine-rich repeat kinase 2 (LRRK2),[64] and had antioxidant effects,[65] suggesting that they could prevent or delay the onset of PD. Clinical studies have provided some support for such an effect; for example, analysis of a cohort of 1,741 participants with early PD found an older mean age of onset of PD in those who reported B12 and multivitamin supplementation prior to PD diagnosis compared to those who reported no use of supplements.[66] Similarly, studies have found higher homocysteine levels and lower vitamin B12 levels among PD cases than matched controls.[67] However, the cross-sectional design of such studies raises the possibility of reverse causality as an alternative explanation to their findings. For instance, an older age of PD onset may have been detected because older participants are more likely to consume vitamin supplements. Likewise,

high homocysteine and low vitamin B12 levels may be a consequence of the underlying PD pathology and not vice versa.

To our knowledge, only two population-based prospective studies have previously examined the association of folate, vitamin B6, and vitamin B12 intake with the risk of PD, with inconclusive results.[68, 69] Results from these studies may have been affected by insufficient statistical power, use of a single dietary assessment, and insufficient follow-up to adequately account for the long prodromal period of PD. We leveraged data from two large prospective cohort studies with ~30 years of follow-up, the Nurses' Health Study (NHS) and the Health Professionals' Follow-up Study (HPFS), and investigated the association between long-term intake of folate, vitamin B6, and vitamin B12 with the subsequent occurrence of PD.

Methods

Study Population

This investigation used data from two large prospective cohort studies: the NHS and the Health HPFS. The NHS was established in 1976 with an enrollment of 121,700 female registered nurses aged 30 to 55 years from 11 US states. The HPFS was established in 1986 with an enrollment of 51,529 male health professionals aged 40 to 75 years from 50 US states. At enrollment, participants from both cohorts responded to a self-administered questionnaire about their health and lifestyle. Follow-up questionnaires have been sent every 2 years to update information on potential risk factors and newly diagnosed diseases. For the present analyses, the source population were women who returned a food frequency questionnaire (FFQ) in 1984 (when the first comprehensive version was administered to NHS participants) and men who returned an FFQ in 1986. At this time, defined as our baseline, we excluded participants with a

diagnosis of Parkinson's disease (PD), implausible total energy intake (<800 or >4200 kcal/day for men, <600 or >3500 kcal/day for women) or missing dietary information. We additionally excluded participants with an unknown year of birth and those who only returned the baseline questionnaire. The base population comprised 80,965 women and 48,837 men.

Outcome Assessment

The primary outcome of interest was incident PD. The process to confirm newly diagnosed cases in both cohorts is detailed elsewhere.[51] Briefly, we identified potential PD cases by biennial self-report questionnaires and then contacted the treating neurologists to confirm the diagnosis or send a copy of the patients' medical records. Before 2003, cases were confirmed if the treating neurologist or internist considered the diagnosis definite or probable, the medical record included a final diagnosis of PD by a neurologist, or the medical record indicated at least two of three cardinal signs of PD (resting tremor, rigidity, and bradykinesia) in the absence of evidence for other diagnoses. Since 2003, the PD diagnoses were confirmed by review of the medical records conducted by a neurologist specialized in movement disorders (A.Y.H. and M.A.S.).

Assessment of Diet and Other Covariates

Dietary information was collected using a validated semiquantitative Food Frequency Questionnaire (FFQ) sent to participants in 1984, 1986, and every 4 years thereafter. This FFQ includes over 100 items assessing how often in the past year participants typically consume a commonly used portion of foods/beverages. For each item, there are nine possible responses ranging from "never or less than once per month" to "6 or more times per day." The questionnaire also asks about the brands of breakfast cereal (an important source of B vitamins)

and the use of multivitamins. The consumption frequency of each food is then multiplied by the nutrient content of the portion specified to obtain nutrient intakes. Food composition values of folate, vitamin B6, vitamin B12, and other nutrients were obtained from the Harvard University Food Composition Database, derived from the US Department of Agriculture and other sources; these sources have been continually updated, with incorporation of changes in food folate content after grain folate fortification. In a recent validation study in the NHS,[70] the Spearman correlation coefficient between the FFQ estimates and two 7-day dietary record-based estimates was 0.71 for folate, 0.68 for vitamin B6 and 0.68 for vitamin B12 (similar correlation coefficients were observed in the HPFS).[71] Furthermore, studies conducted in these and other cohorts have found that the FFQ predicts circulating levels of folate and vitamin B6.[71-74]

Information on other covariates of interest was collected at baseline and on subsequent questionnaires in both cohorts. These covariates included age, body mass index (BMI), smoking (pack-years), physical activity (metabolic equivalents from recreational and leisure-time activities), alcohol intake, caffeine intake, post-menopausal hormone use (only women), total energy intake, flavonoids intake, dairy intake and a score indicating the degree of adherence to the Mediterranean diet.[75] Covariates were selected based on subject-matter knowledge to adjust for confounding.

Statistical Analysis

Participants contributed person-time from the date of returning the baseline FFQ to date of first PD symptoms, death, last completed questionnaire, or end of follow-up (June 2016 for NHS and January 2016 for HPFS), whichever came first. Analyses were stratified by age in months at the start of follow-up and calendar year of the current questionnaire cycle to control

for potential confounding by these 2 factors. Nutrients were adjusted for total energy intake using the residual method to account for differences in energy requirement among individuals.[76] Because PD is a condition that develops insidiously over a long time, we first conducted analyses focusing on long-term nutrient intakes by using the cumulative average intakes of folate, vitamin B6, and vitamin B12 from all available FFQs up to the start of each 2-year follow-up period, categorized into cohort-specific quintiles.[77] For each B vitamin, we used time-varying Cox-regression models to estimate the hazard ratio (HR) of PD and 95% confidence interval (CI) according to quintiles of dietary, supplemental, and total intake, with the lowest quintile as the reference. We also modeled the quintile median values continuously to assess the linear trend across quintiles. We further adjusted models for BMI (<25, 25-30, 30+ kg/m^2), smoking (never, ≤4, 4-24 and ≥25 pack-years), physical activity (quintiles of MET-h/week), alcohol intake (0 to <5, 5 to <10, 10 to <15, 15 to <30 and ≥30 g/day), caffeine intake (quintiles of mg/day), hormone use (premenopausal, no use, current use and past use) and total energy intake (quintiles of kcal/day). We considered other dietary factors in our models including flavonoid intake, dairy intake, and Mediterranean diet score. We used cumulative averages for BMI, physical activity, and intakes of dietary covariates to represent long-term dietary and lifestyle patterns. Information on smoking and hormone use was updated at each follow-up cycle. Missing indicators were assigned at each time period to those who had missing dietary data.[77] Missing indicators were also used for non-dietary covariates with missing values. We pooled estimates from both cohorts and tested for homogeneity using fixed-effect metanalysis.

We conducted a series of secondary and sensitivity analyses. First, to account for the possibility of reverse causation due to the long prodromal phase of PD, we conducted 8-, 12-, 16- and 20-year lagged analyses. Second, we repeated analyses using as exposure the baseline intake

levels and the intake reported on the most recent FFQ before each follow-up cycle to evaluate the impact of early and recent intake, respectively. Third, to further evaluate the temporal relationship between intake of B vitamins and PD, we explored the association between total baseline intake levels and PD in 3 periods: (i) baseline to <1998, (ii) 1998 to <2006, and (iii) 2006 to 2016. The first 2 coincide with the periods before and after the implementation of mandatory folic acid fortification in the United States[78]. Furthermore, we evaluated potential effect measure modification by alcohol consumption (which decreases the absorption of folate and B12),[79] age (because as age increases the absorption of B12 decreases and the risk of PD increases),[59, 79] and smoking (based on previous findings)[69] by comparing models with and without the product term between the potential effect modifier and individual B vitamins, using likelihood ratio tests. Finally, for vitamin B12, we conducted additional analyses excluding supplement users (as these individuals may importantly differ from the rest of the population in terms of dietary and lifestyle factors) and restricting to individuals whose baseline dietary intake was in the 2 lowest quintiles to explore the impact of higher supplemental intake in this subgroup. All statistical tests were 2-sided, and $p < 0.05$ was considered statistically significant. We used SAS 9.4 (SAS Institute) for analyses.

Results

During the follow-up period, a total of 1,426 incident PD cases were identified (687 in women and 739 in men). Baseline characteristics of study participants according to quintiles of intake of vitamin B12 are presented in **Table 3.1**. Compared to participants in the bottom quintile of intake, those in the top quintile were older and had lower consumption of alcohol and caffeine. Women in the highest quintile of intake were also more physically active and more likely to be current hormone users.

Table 3.1. Characteristics of study participants at baseline according to quintiles of intake of vitamin B12, 1984-86

	Women			Men		
	Quintile 1 (n=23,322)	Quintile 3 (n=13,563)	Quintile 5 (n=14,119)	Quintile 1 (n=11,432)	Quintile 3 (n=10,175)	Quintile 5 (n=9,336)
Total B12 intake, mcg/day	4.0 (0.9)	9.5 (1.1)	28.3 (42.7)	4.9 (1.1)	9.9 (0.8)	29.1 (35.5)
Total folate intake, mcg/day	252.8 (92.3)	432.0 (189.9)	587.5 (320.7)	346.7 (120.8)	425.7 (180.8)	727.9 (401.7)
Total B6 intake, mg/day	4.9 (20.2)	9.7 (26.1)	19.9 (42.2)	4.1 (14.7)	6.4 (18.8)	19.8 (40.8)
Age in years*	49.6 (7.2)	50.5 (7.2)	51.8 (6.9)	53.1 (9.9)	54.2 (9.7)	55.0 (9.7)
Physical activity, met-h week[a]	12.5 (18.7)	14.4 (21.4)	16.5 (24.5)	20.8 (24.8)	20.3 (24.4)	21.0 (25.1)
Body mass index categories, %						
BMI <25	58.1	58.8	54.9	48.9	43.8	44.7
BMI 25-30	24.7	24.4	26.7	41.9	45.3	44.1
BMI 30+	12.4	11.8	13.5	7.1	8.5	8.8
Smoking history, pack-years, %						
Never smoked	42.4	45.0	42.2	45.1	44.5	43.4
<=4 pky	9.2	9.9	9.7	3.8	4.1	4.5
5-24 pky	24.4	23.6	24.8	24.3	24.1	23.8
>= 25 pky	22.7	20.1	21.7	21.6	21.9	22.4
Alcohol intake, g/day	7.3 (12.0)	7.4 (12.2)	6.0 (10.1)	12.3 (17.1)	11.3 (15.0)	10.3 (14.2)
Caffeine intake, mg/day	331.1 (239.4)	303.4 (223.8)	303.6 (239.8)	240.2 (255.8)	241.8 (245.7)	234.5 (253.2)
Total flavonoids intake, mg/day	348.4 (347.4)	346.1 (324.5)	357.1 (340.4)	342.2 (291.2)	311.8 (271.8)	324.1 (286.5)
Total dairy intake, servings/day	1.7 (1.2)	2.2 (1.4)	1.9 (1.3)	1.5 (1.1)	2.1 (1.5)	1.9 (1.4)
Mediterranean diet score	3.6 (1.8)	4.1 (1.8)	4.2 (1.8)	4.4 (1.9)	4.3 (1.9)	4.4 (1.9)
Total energy intake, kcal/day	1,678.1 (534.8)	1,894.9 (553.0)	1,607.5 (506.2)	1,909.7 (602.0)	2,065.4 (618.2)	1,901.5 (601.9)
Postmenopausal hormone use, %						
Premenopausal/missing menopause	40.2	39.6	37.4			
No history of hormone use	33.2	30.3	31.1			
Current hormone use	11.6	14.9	15.2			
Past hormone use	12.6	13.0	13.8			

Values are means (SD) unless otherwise specified and are standardized to the age distribution of the study population. [a]Metabolic equivalents from recreational and leisure-time activities. *Value is not age adjusted.

We did not observe an association between total folate intake and risk of PD in any of the analyses (**Figure 3.1 A**). For cumulative average intake, the pooled HR comparing the top and bottom quintiles of intake was 1.15 (95% CI: 0.95, 1.39, p-trend=0.42). This point estimate was attenuated in analysis looking at lagged, baseline, and recent intakes (**Figure 3.1 A**). No associations were found when we looked at intakes from dietary and supplemental sources separately (**Figure 3.1 A; Tables 3.2 and 3.3; Supplemental tables 3.1 and 3.2).**

Total intake of vitamin B6 was not associated with the risk of PD, except in analyses with a 20 year lag, in which higher intake was associated with lower PD risk (**Figure 3. 1 B and Supplemental table 3.1**). In contrast, recent intake and cumulative intake tended to be associated with an increased PD risk (**Figure 3.1 B**). The inverse association in the 20-years lagged analyses was stronger among women **(Supplemental table 3.1**) and for intake from dietary sources (pooled HR comparing top vs. bottom dietary intake = 0.64; 95% CI 0.44, 0.93, p-trend = 0.02). Likewise, neither baseline intake of vitamin B6 (**Table 3.3**), nor intake during specific calendar periods (**Supplemental table 3.3**) were associated with PD risk.

The total cumulative average intake of vitamin B12 was not associated with PD risk (**Figure 3.1 C**). Our analyses, however, suggested a modest inverse association for baseline total B12 with a pooled hazard ratio comparing the top and bottom quintiles of intake of 0.80 (95% CI 0.67, 0.95, p-trend 0.01; **Table 3.3**). The pooled hazard ratio comparing the top and bottom quintiles of intake was 0.77 (95% CI 0.60, 1.01, p=trend 0.02; **Supplemental table 3.1**) in the 20-year lag analysis. An inverse association was also observed when we looked at the impact of baseline intake on PD in the 2006-2016 period (**Supplemental table 3.3**). Intake from both dietary and supplemental sources appeared to contribute to this association.

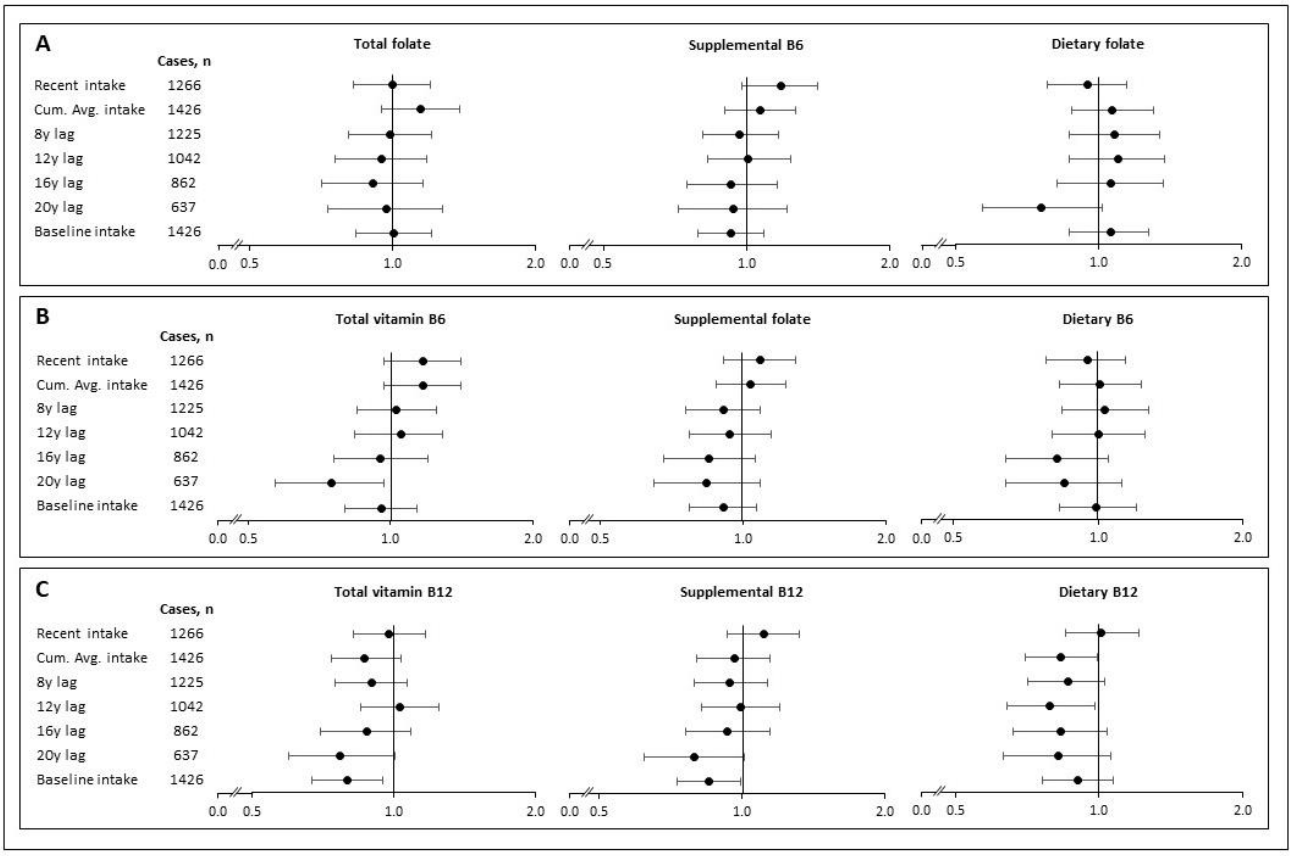


Figure 3.1. Pooled hazard ratios and 95% confidence intervals of Parkinson's disease comparing top to bottom quintiles of B vitamins intake according to different analytical strategies.

Estimates are adjusted for age, calendar time (2-year periods), total energy intake (quintiles) body mass index (<25, 25-30, 30+), smoking (never, ≤4, 4-24 and ≥25 pack-years), alcohol (0 to <5, 5 to <10, 10 to <15, 15 to <30 and ≥30 g/day) and caffeine intake (quintiles), physical activity (quintiles), hormone use (only women; premenopausal, no use, current use and past use), flavonoids intake (quintiles), dairy intake (quintiles) and Mediterranean diet score (quintiles).

Table 3.2. Multivariable adjusted hazard ratios and 95% confidence interval of Parkinson's disease according to cumulative average intake of folate, vitamin B6 and vitamin B12, 1984-86 to 2016

	Women			Men			Pooled
Folate	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3	Model 3
Total							
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	1.37 (1.05, 1.79)	1.34 (1.03, 1.74)	1.32 (1.02, 1.73)	1.11 (0.86, 1.42)	1.09 (0.85, 1.40)	1.07 (0.83, 1.38)	1.18 (0.98, 1.42)
Quintile 3	1.32 (1.02, 1.72)	1.29 (0.99, 1.68)	1.27 (0.97, 1.67)	1.15 (0.90, 1.47)	1.12 (0.87, 1.43)	1.09 (0.84, 1.41)	1.17 (0.97, 1.41)
Quintile 4	1.37 (1.05, 1.78)	1.32 (1.01, 1.72)	1.30 (0.99, 1.71)	1.14 (0.89, 1.4)	1.10 (0.86, 1.42)	1.08 (0.83, 1.40)	1.18 (0.98, 1.42)
Quintile 5	1.30 (1.00, 1.69)	1.25 (0.96, 1.64)	1.23 (0.93, 1.63)	1.17 (0.92, 1.49)	1.11 (0.87, 1.43)	1.09 (0.84, 1.41)	1.15 (0.95, 1.39)
P-trend	*0.16*	*0.28*	*0.39*	*0.33*	*0.56*	*0.72*	*0.42*
P-het							*0.64*
Dietary							
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	1.23 (0.95, 1.59)	1.21 (0.94, 1.56)	1.18 (0.91, 1.53)	0.95 (0.74, 1.23)	0.94 (0.73, 1.21)	0.93 (0.72, 1.21)	1.05 (0.87, 1.26)
Quintile 3	1.12 (0.87, 1.45)	1.09 (0.84, 1.42)	1.05 (0.80, 1.39)	0.94 (0.73, 1.21)	0.91 (0.70, 1.17)	0.90 (0.68, 1.17)	0.97 (0.80, 1.18)
Quintile 4	1.13 (0.87, 1.45)	1.09 (0.84, 1.41)	1.04 (0.78, 1.38)	1.32 (1.04, 1.67)	1.27 (1.00, 1.60)	1.26 (0.97, 1.63)	1.15 (0.95, 1.40)
Quintile 5	1.16 (0.90, 1.50)	1.12 (0.86, 1.45)	1.06 (0.79, 1.42)	1.16 (0.91, 1.48)	1.08 (0.84, 1.38)	1.08 (0.82, 1.42)	1.07 (0.88, 1.31)
P-trend	*0.52*	*0.75*	*0.93*	*0.03*	*0.13*	*0.18*	*0.30*
P-het							*0.39*
Supplemental							
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	1.05 (0.81, 1.35)	1.04 (0.80, 1.34)	1.03 (0.80, 1.33)	1.08 (0.84, 1.38)	1.08 (0.84, 1.39)	1.07 (0.83, 1.37)	1.05 (0.88, 1.25)
Quintile 3	1.03 (0.80, 1.32)	1.01 (0.78, 1.30)	1.00 (0.77, 1.29)	1.02 (0.80, 1.30)	1.03 (0.81, 1.31)	1.01 (0.79, 1.29)	1.00 (0.84, 1.20)
Quintile 4	1.08 (0.85, 1.39)	1.06 (0.83, 1.36)	1.05 (0.82, 1.35)	0.94 (0.74, 1.19)	0.93 (0.73, 1.19)	0.92 (0.72, 1.17)	0.98 (0.82, 1.16)
Quintile 5	1.16 (0.91, 1.48)	1.13 (0.88, 1.44)	1.12 (0.87, 1.42)	1.01 (0.80, 1.26)	1.00 (0.80, 1.26)	0.98 (0.78, 1.24)	1.04 (0.88, 1.23)
P-trend	*0.12*	*0.19*	*0.22*	*0.54*	*0.48*	*0.40*	*0.93*
P-het							*0.14*
Vitamin B6	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3	Model 3
Total							
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	1.45 (1.13, 1.87)	1.42 (1.10, 1.82)	1.40 (1.08, 1.81)	1.25 (0.97, 1.61)	1.23 (0.96, 1.59)	1.21 (0.94, 1.56)	1.30 (1.08, 1.56)
Quintile 3	1.20 (0.92, 1.55)	1.15 (0.89, 1.50)	1.13 (0.87, 1.48)	1.28 (1.00, 1.64)	1.25 (0.97, 1.61)	1.22 (0.95, 1.58)	1.18 (0.98, 1.42)
Quintile 4	1.18 (0.91, 1.53)	1.14 (0.88, 1.49)	1.12 (0.86,1.47)	1.30 (1.02, 1.67)	1.27 (0.99, 1.62)	1.24 (0.96, 1.59)	1.18 (0.98, 1.42)
Quintile 5	1.34 (1.04, 1.74)	1.29 (1.00, 1.68)	1.27 (0.98, 1.66)	1.14 (0.89, 1.47)	1.11 (0.86, 1.43)	1.08 (0.83, 1.41)	1.17 (0.97, 1.41)
P-trend	*0.28*	*0.39*	*0.41*	*0.68*	*0.58*	*0.49*	*0.90*
P-het							*0.29*
Dietary							
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	0.93 (0.71, 1.20)	0.91 (0.70, 1.18)	0.89 (0.68, 1.16)	1.21 (0.93, 1.57)	1.19 (0.91,1.55)	1.18 (0.90, 1.54)	1.02 (0.85, 1.23)
Quintile 3	1.17 (0.92, 1.50)	1.13 (0.88, 1.44)	1.09 (0.84, 1.40)	1.35 (1.05, 1.74)	1.33 (1.03, 1.71)	1.31 (1.00, 1.70)	1.19 (0.99, 1.43)
Quintile 4	1.07 (0.84, 1.36)	1.01 (0.78, 1.29)	0.97 (0.74, 1.26)	1.31 (1.02, 1.68)	1.25 (0.97, 1.62)	1.22 (0.93, 1.59)	1.08 (0.90, 1.31)
Quintile 5	0.94 (0.73, 1.20)	0.87 (0.67, 1.13)	0.83 (0.63, 1.09)	1.36 (1.06, 1.75)	1.26 (0.97, 1.62)	1.23 (0.93, 1.62)	1.01 (0.83, 1.23)
P-trend	*0.78*	*0.36*	*0.20*	*0.03*	*0.20*	*0.35*	*0.98*
P-het							*0.12*
Supplemental							
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	1.14 (0.89, 1.45)	1.12 (0.88, 1.44)	1.11 (0.87, 1.43)	1.14 (0.88, 1.46)	1.15 (0.89, 1.47)	1.14 (0.88, 1.46)	1.12 (0.94, 1.34)
Quintile 3	1.01 (0.79, 1.29)	0.99 (0.77, 1.27)	0.98 (0.76, 1.26)	1.14 (0.89, 1.45)	1.15 (0.90, 1.46)	1.13 (0.89, 1.440	1.05 (0.89, 1.25)
Quintile 4	0.99 (0.77, 1.27)	0.97 (0.76, 1.24)	0.96 (0.75, 1.23)	1.16 (0.91, 1.46)	1.16 (0.92, 1.46)	1.14 (0.90, 1.44)	1.05 (0.89, 1.25)
Quintile 5	1.17 (0.92, 1.49)	1.13 (0.89, 1.45)	1.12 (0.88, 1.44)	1.06 (0.83, 1.34)	1.05 (0.82, 1.33)	1.03 (0.80, 1.31)	1.07 (0.90, 1.27)
P-trend	*0.23*	*0.31*	*0.33*	*0.73*	*0.65*	*0.58*	*0.74*
P-het							*0.28*
Vitamin B12	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3	Model 3
Total							
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	0.93 (0.73, 1.19)	0.92 (0.72, 1.17)	0.91 (0.72, 1.16)	1.04 (0.82, 1.31)	1.04 (0.82, 1.31)	1.02 (0.81, 1.30)	0.97 (0.82, 1.15)
Quintile 3	0.89 (0.70, 1.14)	0.88 (0.69, 1.12)	0.87 (0.68, 1.11)	0.96 (0.76, 1.22)	0.98 (0.78, 1.24)	0.97 (0.76, 1.23)	0.92 (0.77, 1.09)
Quintile 4	0.98 (0.78, 1.24)	0.97 (0.76, 1.23)	0.95 (0.75, 1.21)	1.00 (0.79, 1.26)	1.02 (0.81, 1.29)	1.01 (0.80, 1.27)	0.98 (0.83, 1.16)
Quintile 5	0.88 (0.69, 1.12)	0.87 (0.68, 1.11)	0.86 (0.67, 1.10)	0.90 (0.71, 1.14)	0.90 (0.71, 1.14)	0.89 (0.70, 1.13)	0.87 (0.74, 1.04)
P-trend	*0.45*	*0.49*	*0.51*	*0.72*	*0.67*	*0.61*	*0.97*
P-het							*0.41*
Dietary							
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	0.99 (0.78, 1.25)	0.98 (0.77, 1.25)	0.97 (0.76, 1.24)	0.87 (0.69, 1.11)	0.88 (0.69, 1.11)	0.87 (0.68, 1.10)	0.92 (0.77, 1.09)
Quintile 3	0.99 (0.78, 1.25)	0.98 (0.78, 1.24)	0.97 (0.76, 1.23)	0.88 (0.70, 1.11)	0.89 (0.71, 1.13)	0.88 (0.69, 1.12)	0.92 (0.78, 1.09)
Quintile 4	1.03 (0.82, 1.30)	1.03 (0.82, 1.30)	1.01 (0.80, 1.28)	1.04 (0.83, 1.30)	1.05 (0.84, 1.31)	1.02 (0.81, 1.29)	1.02 (0.86, 1.20)
Quintile 5	0.77 (0.60, 0.99)	0.78 (0.61, 1.01)	0.77 (0.60, 1.00)	0.87 (0.69, 1.09)	0.89 (0.71, 1.13)	0.89 (0.70, 1.12)	0.83 (0.70, 0.99)
P-trend	*0.03*	*0.05*	*0.04*	*0.71*	*0.92*	*0.90*	*0.18*
P-het							*0.11*
Supplemental							
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	1.07 (0.83, 1.37)	1.05 (0.82, 1.34)	1.04 (0.81, 1.34)	1.20 (0.94, 1.52)	1.21 (0.95, 1.53)	1.20 (0.94, 1.52)	1.12 (0.94, 1.33)

Table 3.2 (continued)

Quintile 3	1.01 (0.79, 1.30)	0.99 (0.77, 1.27)	0.98 (0.76, 1.26)	0.99 (0.77, 1.26)	0.99 (0.78, 1.27)	0.98 (0.76, 1.25)	0.98 (0.82, 1.17)
Quintile 4	1.10 (0.87, 1.41)	1.08 (0.85, 1.37)	1.06 (0.83, 1.360	1.11 (0.88, 1.40)	1.13 (0.89, 1.42)	1.11 (0.88, 1.40)	1.09 (0.92, 1.29)
Quintile 5	1.04 (0.81, 1.32)	1.01 (0.80, 1.29)	1.00 (0.78, 1.28)	0.94 (0.74, 1.20)	0.94 (0.74, 1.19)	0.92 (0.72, 1.17)	0.96 (0.80, 1.14)
P-trend	*0.30*	*0.34*	*0.35*	*0.85*	*0.77*	*0.71*	*0.75*
P-het							*0.34*

Model 1: Adjusted for age in months, calendar time (2-year periods) and total energy intake (quintiles).

Model 2: Model 1 + body mass index (<25, 25-30, 30+), smoking (never, ≤4, 4-24 and ≥25 pack-years), alcohol (0 to <5, 5 to <10, 10 to <15, 15 to <30 and ≥30 g/day) and caffeine intake (quintiles), physical activity (quintiles) and hormone use (only women; premenopausal, no use, current use and past use).

Model 3: Model 2 + flavonoids intake (quintiles), dairy intake (quintiles) and Mediterranean diet score (quintiles).

Table 3.3. Multivariable adjusted hazard ratios and 95% confidence interval of Parkinson's disease according to baseline intake of folate, vitamin B6 and vitamin B12, 1984-86 to 2016

	Women			**Men**			Pooled
Folate	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3	Model 3
Total							
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	1.29 (1.01, 1.67)	1.26 (0.98, 1.62)	1.22 (0.94, 1.58)	1.01 (0.79, 1.28)	0.96 (0.76, 1.23)	0.95 (0.74, 1.22)	1.07 (0.90, 1.28)
Quintile 3	1.30 (1.01, 1.67)	1.24 (0.96, 1.59)	1.17 (0.90, 1.53)	1.12 (0.89, 1.42)	1.06 (0.84, 1.34)	1.04 (0.81, 1.34)	1.10 (0.92, 1.32)
Quintile 4	1.17 (0.90, 1.51)	1.11 (0.86, 1.44)	1.04 (0.79, 1.37)	1.05 (0.82, 1.33)	0.97 (0.76, 1.23)	0.96 (0.75, 1.24)	1.00 (0.83, 1.20)
Quintile 5	1.08 (0.83, 1.39)	1.03 (0.79, 1.34)	0.97 (0.73, 1.27)	1.12 (0.89, 1.42)	1.05 (0.82, 1.33)	1.05 (0.81, 1.34)	1.01 (0.84, 1.21)
P-trend	*0.48*	*0.31*	*0.14*	*0.35*	*0.68*	*0.62*	*0.57*
P-het							*0.15*
Dietary							
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	1.03 (0.80, 1.33)	1.00 (0.77, 1.29)	0.97 (0.74, 1.25)	1.27 (1.00, 1.62)	1.24 (0.97, 1.58)	1.22 (0.95, 1.57)	1.09 (0.91, 1.31)
Quintile 3	1.15 (0.90, 1.47)	1.10 (0.86, 1.41)	1.04 (0.80, 1.36)	1.17 (0.92, 1.50)	1.11 (0.87, 1.43)	1.10 (0.85, 1.43)	1.07 (0.89, 1.29)
Quintile 4	1.19 (0.93, 1.52)	1.13 (0.88, 1.44)	1.05 (0.80, 1.38)	1.15 (0.90, 1.47)	1.07 (0.84, 1.38)	1.07 (0.82, 1.40)	1.06 (0.88, 1.28)
Quintile 5	1.05 (0.81, 1.35)	1.00 (0.77, 1.29)	0.92 (0.69, 1.22)	1.30 (1.02, 1.66)	1.18 (0.92, 1.51)	1.20 (0.92, 1.57)	1.06 (0.87, 1.28)
P-trend	*0.59*	*0.88*	*0.57*	*0.12*	*0.51*	*0.45*	*0.79*
P-het							*0.37*
Supplemental							
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	1.04 (0.74, 1.47)	1.05 (0.74, 1.49)	1.06 (0.75, 1.51)	1.23 (0.74, 2.03)	1.24 (0.74, 2.02)	1.21 (0.73, 2.00)	1.11 (0.83, 1.48)
Quintile 3	0.83 (0.57, 1.15)	0.82 (0.59, 1.13)	0.81 (0.59, 1.13)	0.80 (0.59, 1.09)	0.81 (0.59, 1.11)	0.80 (0.59, 1.10)	0.81 (0.64, 1.01)
Quintile 4	0.91 (0.68, 1.22)	0.89 (0.67,1.19)	0.87 (0.65, 1.16)	0.87 (0.68, 1.12)	0.86 (0.67, 1.11)	0.85 (0.66, 1.10)	0.86 (0.71, 1.04)
Quintile 5	0.93 (0.73, 1.19)	0.91 (0.71, 1.17)	0.89 (0.69, 1.14)	0.94 (0.75, 1.16)	0.93 (0.74, 1.15)	0.93 (0.75, 1.16)	0.91 (0.77, 1.07)
P-trend	*0.88*	*0.77*	*0.64*	*0.92*	*0.97*	*0.97*	*0.78*
P-het							*0.70*
Vitamin B6	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3	Model 3
Total							
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	0.96 (0.76, 1.22)	0.94 (0.73, 1.19)	0.91 (0.72, 1.16)	1.14 (0.90, 1.45)	1.12 (0.88, 1.42)	1.09 (0.86, 1.40)	1.00 (0.84, 1.18)
Quintile 3	1.10 (0.86, 1.40)	1.05 (0.82, 1.34)	1.00 (0.78,1.29)	1.07 (0.84, 1.36)	1.01 (0.79, 1.28)	0.98 (0.77, 1.26)	0.99 (0.83, 1.18)
Quintile 4	0.86 (0.67, 1.11)	0.82 (0.64, 1.06)	0.79 (0.61, 1.02)	1.23 (0.98, 1.55)	1.17 (0.93, 1.48)	1.16 (0.91, 1.47)	0.97 (0.81, 1.15)
Quintile 5	1.03 (0.81, 1.31)	1.00 (0.79, 1.27)	0.95 (0.74, 1.22)	1.02 (0.80, 1.29)	0.97 (0.76, 1.23)	0.96 (0.75, 1.23)	0.96 (0.80, 1.14)
P-trend	*0.80*	*0.89*	*0.93*	*0.68*	*0.47*	*0.49*	*0.57*
P-het							*0.68*
Dietary							
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	1.22 (0.97, 1.53)	1.20 (0.95, 1.50)	1.17 (0.93, 1.47)	1.27 (0.98, 1.61)	1.21 (0.95, 1.55)	1.19 (0.93, 1.53)	1.18 (1.00, 1.40)
Quintile 3	1.10 (0.87, 1.40)	1.06 (0.84, 1.35)	1.03 (0.80, 1.31)	1.39 (1.10, 1.77)	1.32 (1.04, 1.69)	1.30 (1.01, 1.66)	1.15 (0.97, 1.37)
Quintile 4	1.13 (0.89, 1.43)	1.08 (0.85, 1.37)	1.03 (0.80, 1.31)	1.06 (0.82, 1.37)	0.97 (0.75, 1.26)	0.95 (0.73, 1.24)	0.99 (0.83, 1.19)
Quintile 5	1.00 (0.78, 1.29)	0.95 (0.73, 1.23)	0.89 (0.68, 1.17)	1.24 (0.97, 1.59)	1.11 (0.86, 1.42)	1.10 (0.84, 1.42)	0.99 (0.82, 1.20)
P-trend	*0.87*	*0.54*	*0.27*	*0.42*	*0.79*	*0.76*	*0.36*
P-het							*0.48*
Supplemental							
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	0.86 (0.65, 1.15)	0.86 (0.64, 1.15)	0.86 (0.64, 1.14)	1.00 (0.74, 1.36)	1.02 (0.75, 1.37)	1.01 (0.74, 1.36)	0.93 (0.75, 1.14)
Quintile 3	0.85 (0.62, 1.16)	0.82 (0.60, 1.13)	0.81 (0.59, 1.11)	1.01 (0.78, 1.32)	1.01 (0.77, 1.31)	0.99 (0.76, 1.29)	0.91 (0.74, 1.12)
Quintile 4	0.84 (0.66, 1.06)	0.82 (0.65, 1.04)	0.80 (0.63, 1.02)	0.99 (0.79, 1.24)	0.99 (0.79, 1.24)	0.98 (0.79, 1.23)	0.89 (0.76, 1.05)
Quintile 5	0.97 (0.77, 1.21)	0.95 (0.76, 1.19)	0.93 (0.74, 1.17)	0.94 (0.76, 1.17)	0.93 (0.75, 1.16)	0.93 (0.75, 1.16)	0.93 (0.79,1.09)
P-trend	*0.73*	*0.78*	*0.92*	*0.52*	*0.45*	*0.47*	*0.66*
P-het							*0.57*
Vitamin B12	Model 1	Model 2	Model 3	Model 1	Model 2	Model 3	Model 3
Total							
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	1.02 (0.81, 1.29)	1.01 (0.80, 1.29)	1.00 (0.79, 1.27)	1.06 (0.84, 1.33)	1.05 (0.83, .32)	1.02 (0.81, 1.28)	1.01 (0.85, 1.19)
Quintile 3	1.09 (0.87, 1.36)	1.07 (0.86, 1.35)	1.05 (0.83, 1.32)	1.15 (0.92, 1.43)	1.15 (0.92, 1.43)	1.11 (0.89, 1.38)	1.08 (0.92, 1.26)
Quintile 4	1.09 (0.86, 1.35)	1.09 (0.88, 1.34)	1.06 (0.86, 1.31)	0.97 (0.77, 1.22)	0.96 (0.76, 1.21)	0.92 (0.73, 1.17)	1.00 (0.85, 1.17)
Quintile 5	0.70 (0.54, 0.91)	0.70 (0.54, 0.90)	0.68 (0.53, 0.88)	0.95 (0.75, 1.20)	0.94 (0.74, 1.18)	0.91 (0.72, 1.14)	0.80 (0.67, 0.95)
P-trend	*0.03*	*0.03*	*0.02*	*0.36*	*0.31*	*0.21*	*0.01*
P-het							*0.38*
Dietary							
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	1.06 (0.83, 1.35)	1.05 (0.82, 1.34)	1.03 (0.81, 1.32)	1.06 (0.84, 1.34)	1.06 (0.84, 1.34)	1.03 (0.82, 1.31)	1.03 (0.87, 1.22)
Quintile 3	1.08 (0.86, 1.37)	1.07 (0.85, 1.36)	1.05 (0.82, 1.33)	1.06 (0.84, 1.33)	1.05 (0.83, 1.32)	1.00 (0.79, 1.27)	1.02 (0.87, 1.21)
Quintile 4	1.22 (0.97, 1.54)	1.22 (0.96, 1.53)	1.19 (0.94, 1.50)	1.10 (0.87, 1.39)	1.10 (0.87, 1.39)	1.04 (0.82, 1.32)	1.11 (0.94, 1.32)
Quintile 5	0.82 (0.64, 1.06)	0.83 (0.64, 1.06)	0.81 (0.63, 1.04)	1.05 (0.83, 1.33)	1.05 (0.83, 1.33)	1.00 (0.78, 1.27)	0.90 (0.76, 1.07)
P-trend	*0.16*	*0.19*	*0.14*	*0.76*	*0.78*	*0.91*	*0.23*

Table 3.3 (continued)

P-het							*0.37*
Supplemental							
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	0.97 (0.76, 1.23)	0.95 (0.75, 1.21)	0.95 (0.74, 1.20)	0.98 (0.76, 1.29)	0.98 (0.76, 1.27)	0.97 (0.75, 1.25)	0.96 (0.80, 1.14)
Quintile 3	0.74 (0.57, 0.96)	0.73 (0.56, 0.95)	0.72 (0.56, 0.93)	1.09 (0.87, 1.37)	1.11 (0.88, 1.39)	1.10 (0.87, 1.38)	0.91 (0.77, 1.08)
Quintile 4	0.77 (0.60, 0.98)	0.76 (0.59, 0.96)	0.74 (0.58, 0.95)	0.99 (0.80, 1.24)	1.00 (0.80, 1.24)	1.00 (0.79, 1.24)	0.87 (0.74, 1.02)
Quintile 5	0.85 (0.69, 1.06)	0.84 (0.67, 1.04)	0.81 (0.65, 1.01)	0.91 (0.73, 1.12)	0.90 (0.72, 1.11)	0.89 (0.72, 1.11)	0.85 (0.73, 0.99)
P-trend	*0.29*	*0.23*	*0.15*	*0.25*	*0.19*	*0.20*	*0.06*
P-het							*0.78*

Model 1: Adjusted for age in months, calendar time (2-year periods) and total energy intake (quintiles).

Model 2: Model 1 + body mass index (<25, 25-30, 30+), smoking (never, ≤4, 4-24 and ≥25 pack-years), alcohol (0 to <5, 5 to <10, 10 to <15, 15 to <30 and ≥30 g/day) and caffeine intake (quintiles), physical activity (quintiles) and hormone use (only women; premenopausal, no use, current use and past use).

Model 3: Model 2 + flavonoids intake (quintiles), dairy intake (quintiles) and Mediterranean diet score (quintiles).

In contrast, we did not observe an association when we looked at the impact of dietary intake of vitamin B12 on PD excluding supplement users at baseline (**Supplemental table 3.4**) or when we evaluated the impact of supplemental intake of B12 among those in the lowest two quintiles of dietary intake at baseline (**Supplemental table 3.5**).

We did not observe evidence of effect modification by baseline alcohol intake (<15g vs ≥15g), age (<50 vs ≥50y for women; <65 vs ≥65y for men) or smoking status (never smokers vs ever smokers), except for total vitamin B12 intake among men (p-value for LR test = 0.05) in which stratified analyses suggested an inverse association among smokers (pooled HR top vs bottom= 0.76; 95% CI 0.53, 1.08, p-trend = 0.06) but not among non-smokers (pooled HR top vs bottom= 1.08; 95% CI 0.78, 1.49, p-trend = 0.78).

Because multivitamins were the main contributor to the intake of B12 in our population (27% of total intake in men and women), as well as one of the main contributors of B6 (16% in women and 21% in men) and folate (25% in both), we further estimated the HR of PD comparing users to nonusers (**Supplemental table 3.6)**. Though not statistically significant, women taking ≥10 pills/wk appeared to have a lower risk of PD, compared to women with no use of multivitamins at baseline (HR=0.72; 95% CI 0.46, 1.13). The rest of the results suggested a null association as did results exploring the combination of use of multivitamins and B complex (**Supplemental table 3.6**).

Finally, to explore the possibility that deficient intakes of folate, vitamin B6 or vitamin B12 are associated with increased PD risk, we further categorized the total intake of these vitamins into deciles and estimated the HR of PD using those in the fifth decile as the reference; no significant results were found among those with the lowest intakes (**Supplemental table 3.7**).

Discussion

In this large prospective study of US health professionals, folate intake was not associated with the risk of Parkinson's disease. Results for vitamin B6 and B12 were less consistent but suggested a modest inverse association, particularly for baseline intake of B12. Recent intake of folate, B6 and B12 supplements tended to be associated with higher PD risk, a trend consistent with reverse causality.

Our investigation is the largest ever conducted on the association between folate, vitamin B6, and B12 and the risk of PD. We took advantage of decades of prospectively collected data on diet, lifestyle, and other risk factors for PD from 129,802 women and men and 1,426 incident PD cases confirmed by specialized physicians. To our knowledge, there are only two population-based longitudinal studies that have previously examined this association.[68, 69] The first study used data from the same cohorts as in our study. However, at the time of this investigation, follow-up data were only available until 2000 (~16 years of follow-up) and 415 PD cases have been identified.[68] Although no statistically significant results were found for the total intake of these 3 vitamins, the authors reported a hazard ratio of 0.6 (95% CI 0.4, 1.0) for women in the highest quintile of total vitamin B12 intake compared to the lowest quintile. Our updated analyses further suggested a modest inverse association which appeared to be slightly stronger among women. The second study was conducted among 5,289 participants from the Rotterdam cohort and identified 74 incident cases over a mean follow-up of 9.7 years.[69] Participants in the highest tertile of total vitamin B6 intake had a lower hazard of PD compared to those in the lowest tertile (HR=0.46; 95% CI 0.22, 0.96); no association was found for folate or vitamin B12. While our 20-year lag analyses provide some support to this finding, most of our results were not consistent with the hypothesis that vitamin B6 lowers the risk of PD.

There are several proposed mechanisms by which folate, vitamin B6 and B12 could have a protective effect on PD. One of these mechanisms is the regulation of high homocysteine levels, which have been neurotoxic in several preclinical studies.[62, 80, 81] B vitamins are known to regulate high homocysteine levels by demethylation to methionine (which requires folate and vitamin B12) and conversion to cysteine (for which vitamin B6 is an essential cofactor). As such, low plasma levels and dietary intakes of these vitamins have been associated with increased plasma homocysteine levels.[82] In addition, vitamin B6 could independently reduce the risk of PD through antioxidant effects unrelated to homocysteine metabolism[65, 83] and through its role in dopamine synthesis.[84] A recent study found that pyridoxine (vitamin B6) supplementation increases the resistance of nigral dopaminergic neurons to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced neurotoxicity in wild-type mice as well as in astrocytic dopamine D2 receptor conditional knockout mice.[65] Finally, recent studies have shown that vitamin B12 inhibits α-synuclein fibrillogenesis[85] and allosterically inhibits the kinase activity of LRRK2,[64] an enzyme whose increased kinase activity resulting from pathogenic gene mutations is implicated in PD pathogenesis.

Our results support the implications of reverse causality when studying risk or protective factors for PD. The prodromal period of PD may begin as early as 20 years before the clinical diagnosis.[86] This period is characterized by the presence of non-motor features including REM-sleep behavior disorder, constipation, olfactory loss, excessive daytime somnolence, urinary dysfunction, depression, bodily pain, and others.[41] In the years preceding the diagnosis, individuals with PD are more likely to see a doctor and to be diagnosed with a variety of conditions.[55, 87] The development of these symptoms could prompt an increased prescription or self-administration of vitamin supplements. This may be especially the case in more health-

conscious populations such as the NHS and HPFS in which the use of supplements is higher than in the general US population.[88] This hypothesis is supported by the trends observed in our study (**Figure 3.1**) in which HRs larger than 1 were observed for recent supplemental vitamin intakes. Another potential explanation for this observation is that only the early intake of B vitamins impacts the risk of PD.

To interpret our findings, several limitations must be considered. First, some degree of measurement error of nutrient intake is inevitable because our measures are based on self-reported intakes and not on actual intakes or blood levels of B vitamins. Yet, the use of repeated assessments reduces some of the random error when reporting diet, and, because of our prospective design, we expect this measurement error to be non-differential with respect to PD, resulting in bias towards the null. Furthermore, previous research suggests that our nutrient assessment method reflects long-term intakes of study participants reasonably well.[70, 71] However, because absorption of B vitamins, in particular for B12, can be affected by a variety of diseases and medications, these results remain limited by a lack of knowledge of blood vitamin levels.[89] Second, given the observational nature of this study, there remains a potential for unmeasured or residual confounding. Third, due to the long follow-up period, selection bias due to differential loss to follow-up or competing events (e.g., death) is possible. Yet, the follow-up rate in our cohorts is close to 90%[90] and it seems unlikely that B vitamins intake could strongly affect dropout or death, decreasing the possibility of such bias. Finally, our study population is predominately Caucasian, highly educated, and well-nourished, which may limit the generalizability of results more broadly (e.g., a population with a deficient intake of B vitamins).

In summary, the results of this large prospective study do not support the hypothesis that increasing folate or vitamin B6 intakes above the current levels would reduce PD risk in this

population of mostly white US health professionals. For vitamin B12, a decreased PD risk was observed among individuals in the highest quintile of intake in some of our analyses, leaving open the possibility of a protective effect of vitamin B12 on the development of PD.

Acknowledgment

The authors would like to thank participants of the Nurses' Health Study and the Health Professionals' Follow-up Study for their participation.

Bibliography

1. Weintraub D, Chahine LM, Hawkins KA, et al. Cognition and the course of prodromal Parkinson's disease. Movement Disorders 2017;32(11):1640-1645.

2. Hely MA, Reid WG, Adena MA, Halliday GM, Morris JG. The Sydney multicenter study of Parkinson's disease: the inevitability of dementia at 20 years. Movement disorders 2008;23(6):837-844.

3. Fengler S, Liepelt-Scarfone I, Brockmann K, Schaffer E, Berg D, Kalbe E. Cognitive changes in prodromal Parkinson's disease: A review. Movement disorders : official journal of the Movement Disorder Society 2017;32(12):1655-1666.

4. Bougea A, Maraki MI, Yannakoulia M, et al. Higher probability of prodromal Parkinson disease is related to lower cognitive performance. Neurology 2019;92(19):e2261-e2272.
5. Aarsland D, Creese B, Politis M, Chaudhuri KR, Weintraub D, Ballard C. Cognitive decline in Parkinson disease. Nature Reviews Neurology 2017;13(4):217.

6. Doty RL, Marcus A, William Lee W. Development of the 12-Item Cross-Cultural Smell Identification Test(CC-SIT). The Laryngoscope 1996;106(3):353-356.

7. Boeve BF, Molano JR, Ferman TJ, et al. Validation of the Mayo Sleep Questionnaire to screen for REM sleep behavior disorder in an aging and dementia cohort. Sleep Med 2011;12(5):445-453.

8. Hughes KC, Gao X, Baker JM, et al. Non-motor features of Parkinson’s disease in a nested case–control study of US men. J Neurol Neurosurg Psychiatry 2018;89(12):1288-1295.

9. Brandt J, Spencer M, Folstein M. The telephone interview for cognitive status. Neuropsychiatry Neuropsychol Behav Neurol 1988;1(2):111-117.

10. Albert M, Smith LA, Scherr PA, Taylor JO, Evans DA, Funkenstein HH. Use of brief cognitive tests to identify individuals in the community with clinically diagnosed Alzheimer's disease. International journal of Neuroscience 1991;57(3-4):167-178.

11. Welsh KA, Butters N, Mohs RC, et al. The Consortium to Establish a Registry for Alzheimer's Disease (CERAD). Part V. A normative study of the neuropsychological battery. Neurology 1994;44(4):609-614.

12. Lezak M. Neumpsychologicalassessment (3rd cd.). New York: Oxford; 1995.

13. Bastug G, Ozel-Kizil ET, Sakarya A, Altintas O, Kirici S, Altunoz U. Oral Trail Making Task as a Discriminative Tool for Different Levels of Cognitive Impairment and Normal Aging. Archives of Clinical Neuropsychology 2013;28(5):411-417.

14. Weisskopf MG, Grodstein F, Ascherio A. Smoking and cognitive function in Parkinson's disease. Movement disorders : official journal of the Movement Disorder Society 2007;22(5):660-665.

15. Stampfer MJ, Kang JH, Chen J, Cherry R, Grodstein F. Effects of moderate alcohol consumption on cognitive function in women. New England Journal of Medicine 2005;352(3):245-253.

16. Go RC, Duke LW, Harrell LE, et al. Development and validation of a structured telephone interview for dementia assessment (STIDA): the NIMH Genetics Initiative. Journal of geriatric psychiatry and neurology 1997;10(4):161-167.

17. Litvan I, Goldman JG, Tröster AI, et al. Diagnostic criteria for mild cognitive impairment in Parkinson's disease: Movement Disorder Society Task Force guidelines. Movement Disorders 2012;27(3):349-356.

18. Desmond DW, Tatemichi TK, Hanzawa L. The Telephone Interview for Cognitive Status (TICS): reliability and validity in a stroke sample. International journal of geriatric psychiatry 1994;9(10):803-807.

19. Pausch C, Schomburg R, Wagenpfeil S, et al. Neuropsychological impairment in prodromal Parkinson's disease. Journal of the neurological sciences 2016;371:117-120.

20. Darweesh SK, Verlinden VJ, Stricker BH, Hofman A, Koudstaal PJ, Ikram MA. Trajectories of prediagnostic functioning in Parkinson's disease. Brain : a journal of neurology 2017;140(2):429-441.

21. Darweesh SKL, Wolters FJ, Postuma RB, et al. Association Between Poor Cognitive Functioning and Risk of Incident Parkinsonism: The Rotterdam Study. JAMA Neurol 2017;74(12):1431-1438.

22. Chahine LM, Weintraub D, Hawkins KA, et al. Cognition in individuals at risk for Parkinson's: Parkinson associated risk syndrome (PARS) study findings. Movement disorders : official journal of the Movement Disorder Society 2016;31(1):86-94.

23. Weintraub D, Simuni T, Caspell-Garcia C, et al. Cognitive performance and neuropsychiatric symptoms in early, untreated Parkinson's disease. Movement disorders : official journal of the Movement Disorder Society 2015;30(7):919-927.

24. Gaenslen A, Wurster I, Brockmann K, et al. Prodromal features for Parkinson's disease--baseline data from the TREND study. European journal of neurology 2014;21(5):766-772.

25. Schrag A, Horsfall L, Walters K, Noyce A, Petersen I. Prediagnostic presentations of Parkinson's disease in primary care: a case-control study. The Lancet Neurology 2015;14(1):57-64.

26. Morley JF, Weintraub D, Mamikonyan E, Moberg PJ, Siderowf AD, Duda JE. Olfactory dysfunction is associated with neuropsychiatric manifestations in Parkinson's disease. Movement disorders : official journal of the Movement Disorder Society 2011;26(11):2051-2057.

27. Takeda A, Baba T, Kikuchi A, et al. Olfactory dysfunction and dementia in Parkinson's disease. J Parkinsons Dis 2014;4(2):181-187.

28. Doty RL. Olfaction in Parkinson's disease and related disorders. Neurobiol Dis 2012;46(3):527-552.

29. Driver-Dunckley E, Adler CH, Hentz JG, et al. Olfactory dysfunction in incidental Lewy body disease and Parkinson's disease. Parkinsonism & Related Disorders;20(11):1260-1262.

30. Vendette M, Montplaisir J, Gosselin N, et al. Brain perfusion anomalies in rapid eye movement sleep behavior disorder with mild cognitive impairment. Movement disorders : official journal of the Movement Disorder Society 2012;27(10):1255-1261.

31. Szeto JYY, Halliday GM, Naismith SL, Lewis SJG. Exploring the Phenotype in Mild Cognitive Impairment to Aid the Prediction of Those at Risk of Transitioning to Parkinson Disease and Dementia With Lewy Bodies. J Geriatr Psychiatry Neurol 2017;30(4):196-205.

32. Ferman TJ, Smith GE, Kantarci K, et al. Nonamnestic mild cognitive impairment progresses to dementia with Lewy bodies. Neurology 2013;81(23):2032-2038.

33. Vann Jones SA, O'Brien JT. The prevalence and incidence of dementia with Lewy bodies: a systematic review of population and clinical studies. Psychological medicine 2014;44(4):673-683.

34. Jonker C, Geerlings MI, Schmand B. Are memory complaints predictive for dementia? A review of clinical and population-based studies. Int J Geriatr Psychiatry 2000;15(11):983-991.

35. Jessen F, Amariglio RE, van Boxtel M, et al. A conceptual framework for research on subjective cognitive decline in preclinical Alzheimer's disease. Alzheimer's & dementia : the journal of the Alzheimer's Association 2014;10(6):844-852.

36. Samieri C, Proust-Lima C, M MG, et al. Subjective cognitive concerns, episodic memory, and the APOE ε4 allele. Alzheimer's & dementia : the journal of the Alzheimer's Association 2014;10(6):752-759.e751.

37. Mitchell AJ, Beaumont H, Ferguson D, Yadegarfar M, Stubbs B. Risk of dementia and mild cognitive impairment in older people with subjective memory complaints: meta-analysis. Acta psychiatrica Scandinavica 2014;130(6):439-451.

38. Molinuevo JL, Rabin LA, Amariglio R, et al. Implementation of subjective cognitive decline criteria in research studies. Alzheimer's & dementia : the journal of the Alzheimer's Association 2017;13(3):296-311.

39. Aarsland D, Creese B, Politis M, et al. Cognitive decline in Parkinson disease. Nature reviews Neurology 2017;13(4):217-231.

40. Fengler S, Liepelt-Scarfone I, Brockmann K, Schäffer E, Berg D, Kalbe E. Cognitive changes in prodromal Parkinson's disease: A review. Movement disorders : official journal of the Movement Disorder Society 2017;32(12):1655-1666.

41. Heinzel S, Berg D, Gasser T, Chen H, Yao C, Postuma RB. Update of the MDS research criteria for prodromal Parkinson's disease. Movement disorders : official journal of the Movement Disorder Society 2019;34(10):1464-1470.

42. Hughes KC, Gao X, Baker JM, et al. Non-Motor Features of Parkinson's Disease in Women. Journal of Parkinson's disease 2021;11(3):1237-1246.

43. Go RC, Duke LW, Harrell LE, et al. Development and validation of a Structured Telephone Interview for Dementia Assessment (STIDA): the NIMH Genetics Initiative. Journal of geriatric psychiatry and neurology 1997;10(4):161-167.

44. Yuan C, Fondell E, Bhushan A, et al. Long-term intake of vegetables and fruits and subjective cognitive function in US men. Neurology 2019;92(1):e63-e75.

45. Caselli RJ, Dueck AC, Osborne D, et al. Longitudinal modeling of age-related memory decline and the APOE epsilon4 effect. The New England journal of medicine 2009;361(3):255-263.

46. Stampfer MJ, Kang JH, Chen J, Cherry R, Grodstein F. Effects of moderate alcohol consumption on cognitive function in women. The New England journal of medicine 2005;352(3):245-253.

47. Litvan I, Goldman JG, Tröster AI, et al. Diagnostic criteria for mild cognitive impairment in Parkinson's disease: Movement Disorder Society Task Force guidelines. Movement disorders : official journal of the Movement Disorder Society 2012;27(3):349-356.

48. Doty RL, Marcus A, Lee WW. Development of the 12-item Cross-Cultural Smell Identification Test (CC-SIT). The Laryngoscope 1996;106(3 Pt 1):353-356.

49. Gao X, Chen H, Schwarzschild MA, Ascherio A. A prospective study of bowel movement frequency and risk of Parkinson's disease. American journal of epidemiology 2011;174(5):546-551.

50. Heinzel S, Berg D, Gasser T, et al. Update of the MDS research criteria for prodromal Parkinson's disease. Movement Disorders 2019;34(10):1464-1470.

51. Gao X, Cassidy A, Schwarzschild M, Rimm EB, Ascherio A. Habitual intake of dietary flavonoids and risk of Parkinson disease. Neurology 2012;78(15):1138-1145.

52. Berwick DM, Murphy JM, Goldman PA, Ware JE, Jr., Barsky AJ, Weinstein MC. Performance of a five-item mental health screening test. Medical care 1991;29(2):169-176.

53. Foubert-Samier A, Helmer C, Le Goff M, et al. Cognitive and functional changes in prediagnostic phase of Parkinson disease: A population-based study. Parkinsonism & related disorders 2020;79:40-46.

54. Schrag A, Anastasiou Z, Ambler G, Noyce A, Walters K. Predicting diagnosis of Parkinson's disease: A risk algorithm based on primary care presentations. Movement Disorders 2019;34(4):480-486.

55. Simonet C, Bestwick J, Jitlal M, et al. Assessment of Risk Factors and Early Presentations of Parkinson Disease in Primary Care in a Diverse UK Population. JAMA Neurology 2022;79(4):359-369.

56. Flores-Torres MH, Hughes KC, Molsberry S, et al. Cognitive function in men with non-motor features of Parkinson's disease. BMJ Neurology Open 2021;3(1):e000112.

57. Doty RL. Olfactory dysfunction in Parkinson disease. Nature Reviews Neurology 2012;8(6):329-339.

58. Driver-Dunckley E, Adler CH, Hentz JG, et al. Olfactory dysfunction in incidental Lewy body disease and Parkinson's disease. Parkinsonism & related disorders 2014;20(11):1260-1262.

59. Bloem BR, Okun MS, Klein C. Parkinson's disease. Lancet (London, England) 2021;397(10291):2284-2303.

60. Schapira AHV, Chaudhuri KR, Jenner P. Non-motor features of Parkinson disease. Nature reviews Neuroscience 2017;18(7):435-450.

61. Kowal SL, Dall TM, Chakrabarti R, Storm MV, Jain A. The current and projected economic burden of Parkinson's disease in the United States. Movement disorders : official journal of the Movement Disorder Society 2013;28(3):311-318.

62. Duan W, Ladenheim B, Cutler RG, Kruman, II, Cadet JL, Mattson MP. Dietary folate deficiency and elevated homocysteine levels endanger dopaminergic neurons in models of Parkinson's disease. Journal of neurochemistry 2002;80(1):101-110.

63. Dakshinamurti K, Paulose C, Siow Y. Neurobiology of pyridoxine. 1985.

64. Schaffner A, Li X, Gomez-Llorente Y, et al. Vitamin B12 modulates Parkinson's disease LRRK2 kinase activity through allosteric regulation and confers neuroprotection. Cell research 2019;29(4):313-329.

65. Wei Y, Lu M, Mei M, et al. Pyridoxine induces glutathione synthesis via PKM2-mediated Nrf2 transactivation and confers neuroprotection. Nature communications 2020;11(1):1-12.

66. Dietiker C, Kim S, Zhang Y, Christine CW. Characterization of Vitamin B12 Supplementation and Correlation with Clinical Outcomes in a Large Longitudinal Study of Early Parkinson's Disease. Journal of movement disorders 2019;12(2):91-96.

67. Shen L. Associations between B vitamins and Parkinson's disease. Nutrients 2015;7(9):7197-7208.

68. Chen H, Zhang SM, Schwarzschild MA, et al. Folate intake and risk of Parkinson's disease. American journal of epidemiology 2004;160(4):368-375.

69. De Lau L, Koudstaal P, Witteman J, Hofman A, Breteler M. Dietary folate, vitamin B12, and vitamin B6 and the risk of Parkinson disease. Neurology 2006;67(2):315-318.

70. Yuan C, Spiegelman D, Rimm EB, et al. Validity of a Dietary Questionnaire Assessed by Comparison With Multiple Weighed Dietary Records or 24-Hour Recalls. Am J Epidemiol 2017;185(7):570-584.

71. Al-Shaar L, Yuan C, Rosner B, et al. Reproducibility and Validity of a Semiquantitative Food Frequency Questionnaire in Men Assessed by Multiple Methods. American Journal of Epidemiology 2020;190(6):1122-1132.

72. Rimm EB, Willett WC, Hu FB, et al. Folate and vitamin B6 from diet and supplements in relation to risk of coronary heart disease among women. Jama 1998;279(5):359-364.

73. Giovannucci E, Stampfer MJ, Colditz GA, et al. Multivitamin use, folate, and colon cancer in women in the Nurses' Health Study. Annals of internal medicine 1998;129(7):517-524.

74. Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. Jama 1993;270(22):2693-2698.

75. Fung TT, McCullough ML, Newby P, et al. Diet-quality scores and plasma concentrations of markers of inflammation and endothelial dysfunction–. The American journal of clinical nutrition 2005;82(1):163-173.

76. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. American journal of epidemiology 1986;124(1):17-27.

77. Hu FB, Stampfer MJ, Rimm E, et al. Dietary fat and coronary heart disease: a comparison of approaches for adjusting for total energy intake and modeling repeated dietary measurements. American journal of epidemiology 1999;149(6):531-540.

78. Food, Administration D. Food standards: Amendment of standards of identity for enriched grain products to require addition of folic acid; final rule (21 CFR Parts 136, 137, and 139). Federal Register 1996;61:8781-8797.

79. Stephenson T, and Wendy J. Schiff. Human Nutrition: Science for Healthy Living. United States: McGraw Hill US Higher Ed USE, 2018.

80. Kruman, II, Culmsee C, Chan SL, et al. Homocysteine elicits a DNA damage response in neurons that promotes apoptosis and hypersensitivity to excitotoxicity. The Journal of neuroscience : the official journal of the Society for Neuroscience 2000;20(18):6920-6926.

81. Imamura K, Takeshima T, Nakaso K, Nakashima K. Homocysteine is toxic for dopaminergic neurons in primary mesencephalic culture. Neuroreport 2007;18(13):1319-1322.

82. Refsum H, Smith AD, Ueland PM, et al. Facts and recommendations about total homocysteine determinations: an expert opinion. Clinical chemistry 2004;50(1):3-32.

83. Mahfouz MM, Kummerow FA. Vitamin C or Vitamin B6 supplementation prevent the oxidative stress and decrease of prostacyclin generation in homocysteinemic rats. The international journal of biochemistry & cell biology 2004;36(10):1919-1932.

84. Peraza AV, Guzmán DC, Brizuela NO, et al. Riboflavin and pyridoxine restore dopamine levels and reduce oxidative stress in brain of rats. BMC Neuroscience 2018;19(1):71.

85. Jia L, Wang Y, Wei W, Zhao W, Lu F, Liu F. Vitamin B12 inhibits α-synuclein fibrillogenesis and protects against amyloid-induced cytotoxicity. Food & function 2019;10(5):2861-2870.

86. Fereshtehnejad SM, Yao C, Pelletier A, Montplaisir JY, Gagnon JF, Postuma RB. Evolution of prodromal Parkinson's disease and dementia with Lewy bodies: a prospective study. Brain : a journal of neurology 2019;142(7):2051-2067.

87. Plouvier AOA, Hameleers RJMG, van den Heuvel EAJ, et al. Prodromal symptoms and early detection of Parkinson’s disease in general practice: a nested case-control study. Family Practice 2014;31(4):373-378.

88. Kim HJ, Giovannucci E, Rosner B, Willett WC, Cho E. Longitudinal and secular trends in dietary supplement use: Nurses' Health Study and Health Professionals Follow-Up Study, 1986-2006. Journal of the Academy of Nutrition and Dietetics 2014;114(3):436-443.

89. Green R, Allen LH, Bjørke-Monsen A-L, et al. Vitamin B12 deficiency. Nature Reviews Disease Primers 2017;3(1):17040.

90. Bao Y, Bertoia ML, Lenart EB, et al. Origin, Methods, and Evolution of the Three Nurses' Health Studies. American journal of public health 2016;106(9):1573-1581.

Appendix 1: Supplemental Tables for Chapter 1

Supplemental table 1.1. Tests of cognitive function in participants of the HPFS-ProPD study.

Test	Latent domain	Task description	n	Mean (SD)					
				Prodromal features					PD
				No features	Hyposmia	Hyposmia + constipation	Hyposmia + pRBD	Hyposmia, constipation, pRBD	
TICS	"Global cognition"	Modeled on the Mini-Mental State Examination. Scores range from 0 to 42.	803	33.0 (2.5)	32.4 (3.6)	31.8 (3.3)	32.4 (3.4)	31.7 (3.7)	30.5 (4.0)
EBMT									
Immediate recall	Memory	A short story is read to the respondent. Twelve key elements must be repeated immediately. Scores range from 0 to 12.	787	9.4 (1.8)	8.9 (1.9)	9.1 (1.7)	8.8 (1.8)	8.6 (1.8)	8.4 (1.4)
Delayed recall	Memory	A test of delayed recall is given 15 min later asking for the same 12 key elements.	786	8.8 (2.2)	7.9 (3.0)	8.4 (2.2)	8.1 (2.4)	8.0 (2.5)	7.7 (2.4)
TICS 10-word list, immediate recall	Memory	A ten-word list is read to respondent as part of the TICS. These words must be repeated immediately; scores range from 0 to 10.	803	3.9 (1.5)	3.8 (1.8)	3.4 (1.5)	3.7 (1.6)	3.5 (1.7)	3.1 (1.4)
TICS 10-word list, delayed recall	Memory	Participants are asked to recall a ten-word list previously asked to repeat during the TICS; scores range from 0 to 10.	802	1.8 (1.7)	1.4 (1.8)	1.3 (1.7)	1.5 (1.5)	1.3 (1.6)	1.2 (1.3)
Digit span backwards	Executive function/	Sequences of digits are read to the respondent who must recall them in backward order. Scores range from 0 to 12.	801	6.8 (2.6)	6.3 (2.3)	6.5 (2.4)	6.4 (2.3)	6.7 (2.5)	5.9 (2.5)
OTMT-A	Processing Speed/ Attention	Participants are asked to count numbers from 1 to 25. The application time is recorded by a chronometer. If there are two errors, or time goes over 2 minutes, time is recorded as "120".	804	10.7 (3.0)	11.9 (7.9)	12.4 (4.4)	11.3 (4.1)	12.5 (10.4)	14.0 (16.6)
OTMT-B	Executive function/	Participants are asked to count to 13 and state the alphabet in alternating order. The application process is the same as for the OTMT-A.	803	42.9 (27.8)	47.0 (28.7)	54.3 (32.8)	49.4 (33.4)	57.2 (36.6)	69.7 (41.1)

Supplemental table 1.1. (continued)									
Animal naming test	Language/ Verbal fluency	Participants name as many animals as they can during 1 min; their score is the number of animals named.	802	18.8 (5.1)	17.6 (5.9)	16.4 (5.1)	16.8 (5.8)	16.0 (5.4)	13.8 (5.4)

TICS, Telephone Interview for Cognitive Status; EBMT, East Boston Memory Test; OTMT, Oral Trail Making Test.

Supplemental table 1.2. Adjusted mean z scores differences and 95% confidence interval according to prodromal features and confirmed PD.

Measure of cognitive function	**n**	**Prodromal features**				**Confirmed PD**
		No features	Hyposmia only	Hyposmia + constipation	Hyposmia + pRBD	Hyposmia, constipation, pRBD	
		Mean z score differences (95 percent confidence interval)					
Global cognitive score	771						
Age-adjusted		ref	-0.28 (-0.49, -0.07)	-0.33 (-0.55, -0.10)	-0.31 (-0.54, -0.09)	-0.48 (-0.72, -0.25)	-0.84 (-1.18, -0.50)
Multivariate-adjusted		ref	-0.30 (-0.50, -0.09)	-0.32 (-0.55, -0.10)	-0.32 (-0.55, -0.09)	-0.46 (-0.70, -0.22)	-0.80 (-1.15, -0.47)
TICS score	803						
Age-adjusted		ref	-0.11 (-0.32, 0.09)	-0.27 (-0.49, -0.04)	-0.17 (-0.39, 0.06)	-0.32 (-0.55, -0.08)	-0.79 (-1.11, -0.47)
Multivariate-adjusted		ref	-0.13 (-0.33, 0.08)	-0.26 (-0.48, -0.03)	-0.16 (-0.38, 0.07)	-0.29 (-0.52, -0.05)	-0.75 (-1.07, -0.43)
TICS immediate 10-word list	803						
Age-adjusted		ref	0.01 (-0.20, 0.22)	-0.21 (-0.43, 0.01)	-0.16 (-0.39, 0.06)	-0.19 (-0.42, 0.05)	-0.55 (-0.87, -0.23)
Multivariate-adjusted		ref	0.00 (-0.21, 0.20)	-0.20 (-0.43, 0.02)	-0.16 (-0.39, 0.06)	-0.15 (-0.39, 0.09)	-0.53 (-0.85, -0.21)
TICS delayed 10-word list	802						
Age-adjusted		ref	-0.16 (-0.37, 0.05)	-0.21 (-0.44, 0.02)	-0.18 (-0.41, 0.04)	-0.24 (-0.48, 0.00)	-0.43 (-0.75. -0.10)

Supplemental table 1.2 (continued)							
Multivariate-adjusted		ref	-0.17 (-0.39, 0.04)	-0.21 (-0.44, 0.01)	-0.20 (-0.43, 0.03)	-0.24 (-0.49, 0.00)	-0.44 (-0.77, -0.11)
EBMT							
Immediate recall	787						
Age-adjusted		ref	-0.25 (-0.46, -0.03)	-0.12 (-0.36, 0.11)	-0.34 (-0.57, -0.11)	-0.42 (-0.67, -0.18)	-0.63 (-0.97, -0.29)
Multivariate-adjusted		ref	-0.26 (-0.48, -0.05)	-0.12 (-0.36, 0.11)	-0.35 (-0.59, -0.12)	-0.40 (-0.65, -0.15)	-0.60 (-0.94, -0.26)
Delayed recall	786						
Age-adjusted		ref	-0.30 (-0.51, -0.08)	-0.12 (-0.35, 0.12)	-0.26 (-0.49, -0.02)	-0.29 (-0.54, -0.04)	-0.45 (-0.79, -0.11)
Multivariate-adjusted		ref	-0.30 (-0.52, -0.08)	-0.11 (-0.34, 0.13)	-0.26 (-0.50, -0.02)	-0.28 (-0.53, -0.03)	-0.44 (-0.79, -0.10)
Digit span backwards	801						
Age-adjusted		ref	-0.19 (-0.40, 0.03)	-0.09 (-0.33, 0.15)	-0.16 (-0.39, 0.08)	-0.01 (-0.26, 0.24)	-0.41 (-0.75, -0.07)
Multivariate-adjusted		ref	-0.20 (-0.42, 0.02)	-0.10 (-0.34, 0.14)	-0.17 (-0.41, 0.07)	-0.02 (-0.27, 0.23)	-0.41 (-0.75, -0.06)
OTMT-B	803						
Age-adjusted		ref	-0.10 (-0.32, 0.11)	-0.32 (-0.55, -0.09)	-0.21 (-0.44, 0.03)	-0.42 (-0.67, -0.18)	-0.85 (-1.19, -0.51)
Multivariate-adjusted		ref	-0.11 (-0.32, 0.11)	-0.33 (-0.56, -0.09)	-0.22 (-0.45, 0.02)	-0.44 (-0.69, -0.19)	-0.87 (-1.21, -0.53)
OTMT-A	804						
Age-adjusted		ref	-0.14 (-0.36, 0.08)	-0.21 (-0.44, 0.03)	-0.08 (-0.32, 0.16)	-0.23 (-0.48, 0.02)	-0.45 (-0.79, -0.11)
Multivariate-adjusted		ref	-0.14 (-0.36, 0.08)	-0.20 (-0.43, 0.04)	-0.06 (-0.30, 0.18)	-0.22 (-0.47, 0.03)	-0.43 (-0.77, -0.09)
Animal naming test	802						

Supplemental table 1.2 (continued)

Age-adjusted	ref	-0.18 (-0.38, 0.03)	-0.37 (-0.60, -0.15)	-0.37 (-0.60, -0.14)	-0.48 (-0.71, -0.24)	-0.97 (-1.29, -0.65)
Multivariate-adjusted	ref	-0.18 (-0.39, 0.03)	-0.37 (-0.59, -0.14)	-0.36 (-0.59, -0.14)	-0.46 (-0.70, -0.22)	-0.94 (-1.26, -0.61)

*TICS, Telephone Interview for Cognitive Status; EBMT, East Boston Memory Test; OTMT, Oral Trail Making Test. The global score is a composite of all cognitive tests. Multivariate-adjusted models were adjusted for age (years, continuous), physical activity (met-h/week, quartiles), body-mass index (normal weight, overweight, and obese), smoking status (never, ever, unknown), alcohol consumption (g/day, continuous) and coffee consumption (servings/day, continuous).

Supplemental table 1.3. Adjusted odds ratios of subjective cognitive decline items according to presence of prodromal features and confirmed PD.

		Prodromal features				Confirmed PD
	No features	Hyposmia only	Hyposmia + constipation	Hyposmia + pRBD	Hyposmia, constipation, and pRBD	
	Odds ratios (95 percent confidence interval)					
Perceived recent change ability to:						
1. Remember things	Ref	1.67 (1.04, 2.68)	1.95 (1.17, 3.25)	2.15 (1.29, 3.58)	2.4 (1.41, 4.10)	2.51 (1.22, 5.18)
2. Remember recent events	Ref	3.38 (1.58, 7.23)	3.48 (1.57, 7.69)	4.81 (2.21, 10.50)	5.67 (2.58, 12.47)	11.46 (4.57, 28.78)
3. Remember a short list of items	Ref	1.03 (0.62, 1.72)	1.46 (0.85, 2.49)	1.62 (0.95, 2.76)	1.94 (1.11, 3.37)	3.62 (173, 7.55)
4. Remember things from one second to the next	Ref	1.03 (0.56, 1.92)	1.31 (0.68, 2.50)	1.98 (1.06, 3.69)	1.65 (0.85, 3.20)	1.57 (0.65, 3.78)
5. Understand or follow spoken instructions	Ref	1.45 (0.64, 3.30)	0.83 (0.32, 2.19)	1.84 (0.78, 4.33)	2.90 (1.25, 6.73)	7.74 (2.99, 20.08)
6. Follow a group conversation or plot of a television program	Ref	1.13 (0.43, 2.93)	2.64 (1.05, 6.61)	3.98 (1.62, 9.79)	3.00 (1.18, 7.62)	7.97 (2.77, 22.94)
7. Find one's way on familiar streets	Ref	2.33 (0.48, 11.28)	2.13 (0.40, 11.36)	2.68 (0.52, 13.70)	6.98 (1.51, 32.35)	13.72 (2.68, 70.15)

Multivariate-adjusted 1 included age (years, continuous), physical activity (met-h/week, quartiles), body-mass index (normal weight, overweight, and obese), smoking status (never, ever, unknown), alcohol consumption (g/day, continuous) and coffee consumption (servings/day, continuous). Multivariate-adjusted 2 included all variables from the previous model plus depressive symptoms (yes, no); 16 observations were excluded from the analysis due to missing data on depressive symptoms.

Appendix 2: Supplemental Methods, Tables and Figures for Chapter 2

Supplemental Methods

In addition to hyposmia, constipation and pRBD, we also collected information on 4 additional prodromal markers of PD: excessive daytime somnolence, urinary dysfunction, depression, and abnormal motor symptoms. Excessive daytime somnolence was measured in 2015 using the Epworth Sleepiness Scale and defined as a score ≥10. Information on urinary dysfunction has been collected biennially in the NHS since baseline. For the present study, we used information on assessments from the past 10 years (2004-2014). We defined urinary dysfunction according to the Sandvik severity index which incorporates information on the frequency and amount of involuntary urinary leakage and it is categorized into slight, moderate, and severe urinary dysfunction. The presence of depressive symptoms was assessed using the Mental Health Inventory (MHI) and defined as a score in the bottom 10% of women without pRBD or constipation. To construct the prodromal PD score, we also consider clinician-diagnosed depression or use of antidepressants reported in the last 10 years. We measured the presence of abnormal motor symptoms using a nine-item screening questionnaire that assessed self-reported changes in motor function; the number of changes in motor function were summed to calculate a motor score. Among 51 participants of the NHS and the Health Professionals' Follow-up Study assessed in person by movement disorders specialists, the Spearman correlation coefficient between this score and the MDS-UPDRS part 2 ("Motor experiences of daily living") was 0.82 ($p < 0.0001$), and with part 3 ("Motor examination") was 0.66 ($p < 0.0001$). Reporting at least 4 changes in motor function has been shown to have a sensitivity of 90% and a specificity of 94% for neurologist-confirmed parkinsonism. For the present study, we defined abnormal motor function as a score 1 SD above the mean score among PD-free women without hyposmia, constipation, and pRBD (score > 2.7).

Information on other covariates of interest was obtained from responses to the NHS 2014 general questionnaire or the most recent questionnaire available. These covariates include age, education, physical activity (MET-hrs/wk), body-mass index (kg/m2), smoking (pack-years), alcohol consumption (g/day), caffeine intake (mg/day), and self-reported diagnosis of type 2 diabetes.

Supplemental table 2.1. Age-standardized characteristics of NHS-ProPD participants with and without complete data on covariates

	Incomplete data (n=1,744)	**Complete data** (n=12,427)
Age in years[a]	77.5 (5.1)	77.8 (5.1)
Education level, %		
Associate degree	65.4	64.6
Bachelor's degree	23.7	23.0
Graduate degree	10.9	12.4
Physical activity MET-h/w[b]	22.9 (27.2)	24.3 (26.7)
Body mass index categories, %		
Normal weight (<25 kg/m^2)	41.1	46.3
Overweight (25 to <30 kg/m^2)	36.6	33.8
Obese (≥30 kg/m^2)	22.3	19.9
Alcohol g/d	6.2 (10.6)	6.8 (11.1)
Smoking pack-years, %		
Never smoked	47.5	48.2
<=4 pky	11.9	12.5
5-24 pky	23.2	24.4
>=25 pky	17.4	14.9
Caffeine mg/d	151.5 (148.1)	142.5 (133.4)
Depressive symptoms, %	18.6	16.3
Poor SCD in 2014, %	11.9	11.3
Hyposmia, **%**	19.3	20.2
Hyposmia, constipation and pRBD, %	3.3	4.6

Values are means (standard deviations) unless otherwise specified. [a]Value is not age-adjusted. [b]Metabolic equivalents from recreational and leisure-time activities.

Supplemental table 2.2. Tests of cognitive function in the study population according to the presence of non-motor features of PD, n=670

Test	Latent domain	Task description	No features	Hyposmia				
					+ constipation	+pRBD	+ constipation and pRBD	PD
TICS	"Global cognition"	Modeled on the Mini-Mental State Examination. Scores range from 0 to 42.	33.2 (3.1)	31.6 (4.8)	32.1 (3.7)	32.8 (2.9)	30.7 (3.8)	30.5 (3.2)
TICS 10-word list, immediate recall	Memory	A ten-word list is read to respondent as part of the TICS. These words must be repeated immediately; scores range from 0 to 10.	2.7 (1.7)	3.9 (2.0)	4.2 (1.8)	4.6 (1.7)	3.6 (1.6)	3.5 (1.5)
TICS 10-word list, delayed recall	Memory	Participants are asked to recall a ten-word list previously asked to repeat during the TICS; scores range from 0 to 10.	2.5 (2.0)	2.0 (1.9)	2.2 (2.2)	2.2 (2.2)	1.6 (1.7)	0.9 (1.7)
EBMT								
Immediate recall	Memory	A short story is read to the respondent. Twelve key elements must be repeated immediately. Scores range from 0 to 12.	9.5 (1.8)	8.9 (2.2)	9.2 (1.8)	9.4 (1.8)	9.0 (1.6)	8.8 (1.3)
Delayed recall	Memory	A test of delayed recall is given 15 min later asking for the same 12 key elements.	9.0 (2.7)	8.0 (3.2)	8.3 (3.1)	9.0 (2.2)	8.3 (2.6)	7.8 (2.7)
Digit span backwards	Executive function/ working memory	Sequences of digits are read to the respondent who must recall them in backward order. Scores range from 0 to 12.	7.7 (2.3)	6.5 (2.6)	6.6 (2.6)	6.7 (2.0)	6.1 (2.4)	6.5 (2.1)

Supplemental table 2.2 (continued)

OTMT-A[a]	Processing Speed/ Attention	Participants are asked to count numbers from 1 to 25. The application time is recorded by a chronometer.	107.9 (3.5)	107.5 (8.8)	107.9 (3.5)	107.5 (4.7)	107.7 (3.8)	107.6 (3.2)
OTMT-B[a]	Executive function/ working memory	Participants are asked to count to 13 and state the alphabet in alternating order. The application process is the same as for the OTMT-A.	72.6 (25.9)	61.9 (35.3)	63.7 (33.6)	57.1 (36.7)	52.2 (35.3)	41.9 (38.8)
Animal naming test	Language/ Verbal fluency	Participants name as many animals as they can during 1 min; their score is the number of animals named.	18.0 (5.4)	14.5 (4.9)	15.4 (4.9)	15.9 (4.9)	14.8 (4.5)	13.6 (5.6)

All values are means (standard deviations). TICS, Telephone Interview for Cognitive Status; EBMT, East Boston Memory Test; OTMT, Oral Trail Making Test. *Scores are reverse-coded so that higher values indicate better performance.

Supplemental table 2.3. Age-standardized characteristics of women selected for cognitive testing according to response status.

	Non-responders (n=595)	**Responders** (n=715)
Age in years[a]	80.9 (5.2)	79.9 (5.0)
Education level, %		
Associate degree	66.9	56.8
Bachelor's degree	21.5	25.5
Graduate degree	11.6	17.8
Physical activity MET-h/w[b]	17.8 (22.9)	21.5 (23.1)
Body mass index categories, %		
Normal weight (<25 kg/m^2)	51.4	46.4
Overweight (25 to <30 kg/m^2)	34.4	34.5
Obese (≥30 kg/m^2)	14.2	19.0
Alcohol g/d	5.1 (9.0)	7.4 (12.0)
Smoking pack-years, %		
Never smoked	49.7	47.1
<=4 pky	10.2	10.2
5-24 pky	22.0	26.9
>=25 pky	18.1	15.8
Caffeine mg/d	134.4 (140.3)	149.4 (137.4)
Depressive symptoms, %	21.6	15.9
Poor SCD in 2014, %	20.7	13.0
Hyposmia, **%**	89.9	87.4
Hyposmia, constipation and pRBD, %	15.5	12.0

Values are means (standard deviations) unless otherwise specified. 10% had missing data on at least 1 variable. [a]Value is not age-adjusted. [b]Metabolic equivalents from recreational and leisure-time activities.

Supplemental table 2.4. Availability of risk and prodromal markers in the study population				
	LR +	**LR -**	**Available**	**LR assigned**
Risk Markers				
Male sex	1.2	0.8	Yes	0.8 to all
Exposure to pesticides	1.5	N/A (1)	No, but unlikely	1 to all
Exposure to solvents	1.5	N/A (1)	No, but unlikely	1 to all
Nonuse of caffeine	1.35	0.88	Yes, cum. avg. up to 2010 (caffeine from diff. sources)	LR 1.35 if <59mg/d LR 1 if <137mg/d or missing LR 0.88 if ≥137mg/d
Nonsmoking status	1.2 (never) 0.51 (current) 0.91 former)		Yes, most recent 2014	LR 1.2 if never LR 0.51 if current LR 0.91 if former LR 1 if missing
Genetics	2.5-7.5		No	LR 1 to all
Abnormal hyper echogenicity of the SN	4.7	0.45	No	LR 1 to all
Diabetes (type 2)	1.5	0.97	Yes, most recent 2014.	LR 1.5 if self-reported T2DM at any time during follow-up LR 0.97 if no T2DM LR 1 if missing
Physical inactivity	1.3	0.91	Yes, cum. avg. up to 2014	LR 1.3 if ≤ 3 METs hrs/week LR 1 if >3 and ≤ 9 or missing LR 0.91 if >9 met-hrs/week
Low plasma urate levels			NA (only in men)	LR 1 to all
Prodromal Markers				
PSG proven RBD or	130	0.65	No	
Positive response to screening test	2.8	0.89	Yes, 2012 and 2015 1Q	LR 2.8 if pRBD LR 1 if missing LR 0.89 if no pRBD
Olfactory dysfunction	6.4	0.40	Yes, 2015 UPSIT	LR 6.4 If UPSIT <=10% LR 0.4 if >10% LR 1 if missing
Constipation	2.5	0.82	Yes, 2012 and 2015	LR 2.5 if present LR 0.82 if absent LR 1 if missing

Supplemental table 2.4 (continued)				
Excessive daytime somnolence	2.7	0.86	Yes, 2015 Epworth Sleepiness Scale; defined as a score ≥10.	LR 2.7 if present LR 0.86 if absent LR 1 if missing
Symptomatic hypotension	3.2	0.8	No	LR 1 to all
Erectile dysfunction	3.4	0.87	NA	NA
Urinary dysfunction	2.0	0.9	Yes, based on Sandvik severity index	LR 2.0 if severe dysfunction experienced in the last 10 years LR 0.9 if slight or moderate LR 1 if missing
Depression	1.6	0.88	Yes, clinician diagnosed or use of antidepressants in the last 10 years OR MHI score in bottom 10% in 2015	LR 1.6 if present LR 0.88 if absent LR 1 if missing
Global cognitive deficit	1.8	0.88	No	LR 1 to all
Subthreshold parkinsonism	10	0.7		
OR Abnormalities in quantitative motor tests	3.5	0.6	Yes, 9-item screening questionnaire by Tanner et al.	LR 3.5 if score >1SD above mean in those with no hyposmia, constipation and pRBD (and no PD) LR 0.6 if <SD LR 1 if missing
Neuroimaging/Biomarkers				
Abnormal SPECT	40	0.65	No	LR 1 to all

Supplemental table 2.5. Adjusted odds ratios and 95% confidence intervals of cognitive impairment according to presence of prodromal features and diagnosed PD in the subset population.

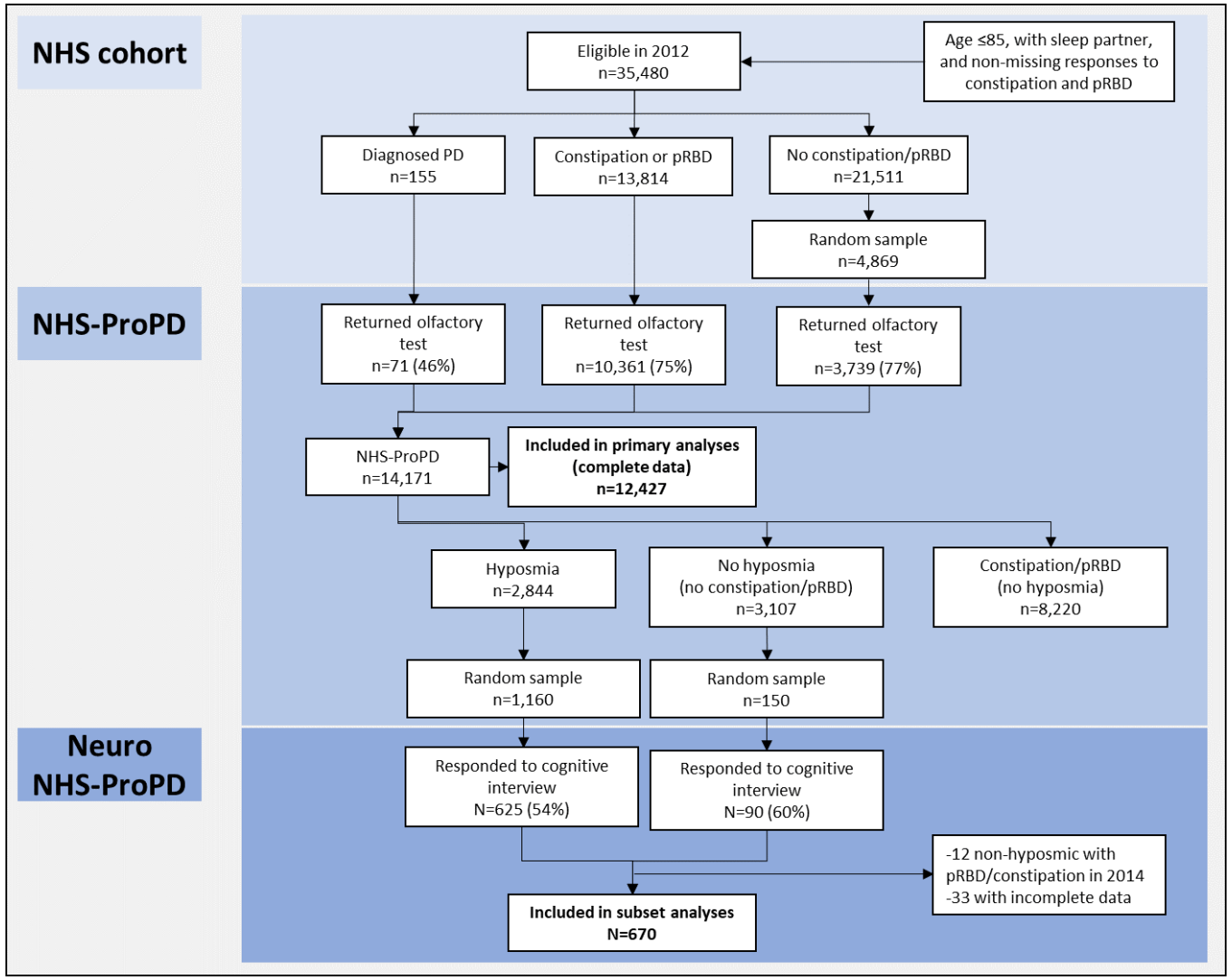
	No features	Hyposmia				
			+ constipation	+ pRBD	+ constipation and pRBD	Diagnosed PD
n	75	178	269	48	80	20
Global cognition						
Age-adjusted	ref	3.91 (1.78, 8.57)	3.74 (1.75, 8.02)	2.97 (1.12, 7.91)	4.80 (2.04, 11.30)	12.98 (3.99, 42.21)
Multivariable	ref	3.34 (1.50, 7.44)	2.83 (1.29, 6.19)	2.40 (0.87, 6.60)	3.92 (1.63, 9.44)	10.92 (3.19, 37.42)
TICS score < 31						
Age-adjusted	ref	2.06 (1.03, 4.15)	2.05 (1.05, 4.02)	1.29 (0.50, 3.32)	3.18 (1.47, 6.88)	4.15 (1.38, 12.50)
Multivariable	ref	1.96 (0.96, 4.03)	1.76 (0.87, 3.53)	1.07 (0.40, 2.85)	2.85 (1.28, 6.32)	3.91 (1.21, 12.61)
Memory						
Age-adjusted	ref	3.54 (1.57, 7.99)	2.61 (1.18, 5.78)	1.99 (0.70, 5.69)	3.47 (1.42, 8.47)	4.65 (1.40, 15.45)
Multivariable	ref	3.50 (1.53, 8.01)	2.36 (1.04, 5.33)	1.90 (0.65, 5.57)	3.21 (1.29, 7.99)	4.80 (1.40, 16.47)
Executive function						
Age-adjusted	ref	3.12 (1.48, 6.59)	2.51 (1.21, 5.20)	4.59 (1.87, 11.27)	5.00 (2.22, 11.26)	8.37 (2.72, 25.78)
Multivariable	ref	2.95 (1.38, 6.28)	2.26 (1.08, 4.75)	4.56 (1.82, 11.42)	4.68 (2.04, 10.75)	8.24 (2.60, 26.18)
Attention						
Age-adjusted	ref	0.82 (0.38, 1.80)	1.21 (0.59, 2.47)	1.75 (0.69, 4.45)	1.23 (0.52, 2.91)	1.44 (0.40, 5.14)
Multivariable	ref	0.75 (0.34, 1.66)	1.04 (0.50, 2.19)	1.42 (0.54, 3.70)	1.03 (0.45, 2.49)	1.18 (0.31, 4.40)

Supplemental table 2.5 (continued)

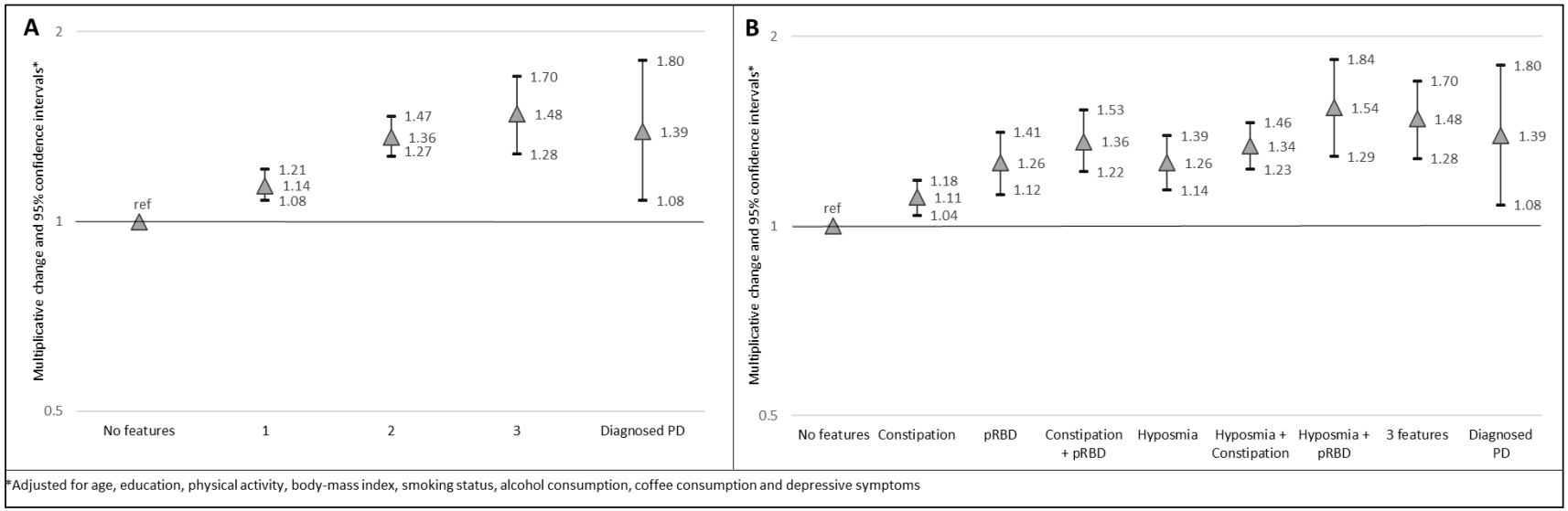
Language						
Age-adjusted	ref	3.25 (1.58, 6.68)	2.90 (1.44, 5.82)	2.24 (0.90, 5.60)	2.68 (1.20, 5.99)	6.01 (1.99, 18.09)
Multivariable-adjusted	ref	3.16 (1.52, 6.57)	2.84 (1.39, 5.83)	2.10 (0.82, 5.37)	2.55 (1.12, 5.82)	6.58 (2.12, 20.44)

Multivariable models were adjusted for age (years, continuous), education (associate degree, bachelor's degree, or graduate degree), physical activity (met-h/week, continuous), body-mass index (normal weight, overweight, or obese), smoking status (never, <5, 5-<25, or ≥25 pack years), alcohol consumption (g/day, continuous), caffeine intake (mg/day, continuous) and depressive symptoms (yes/no).

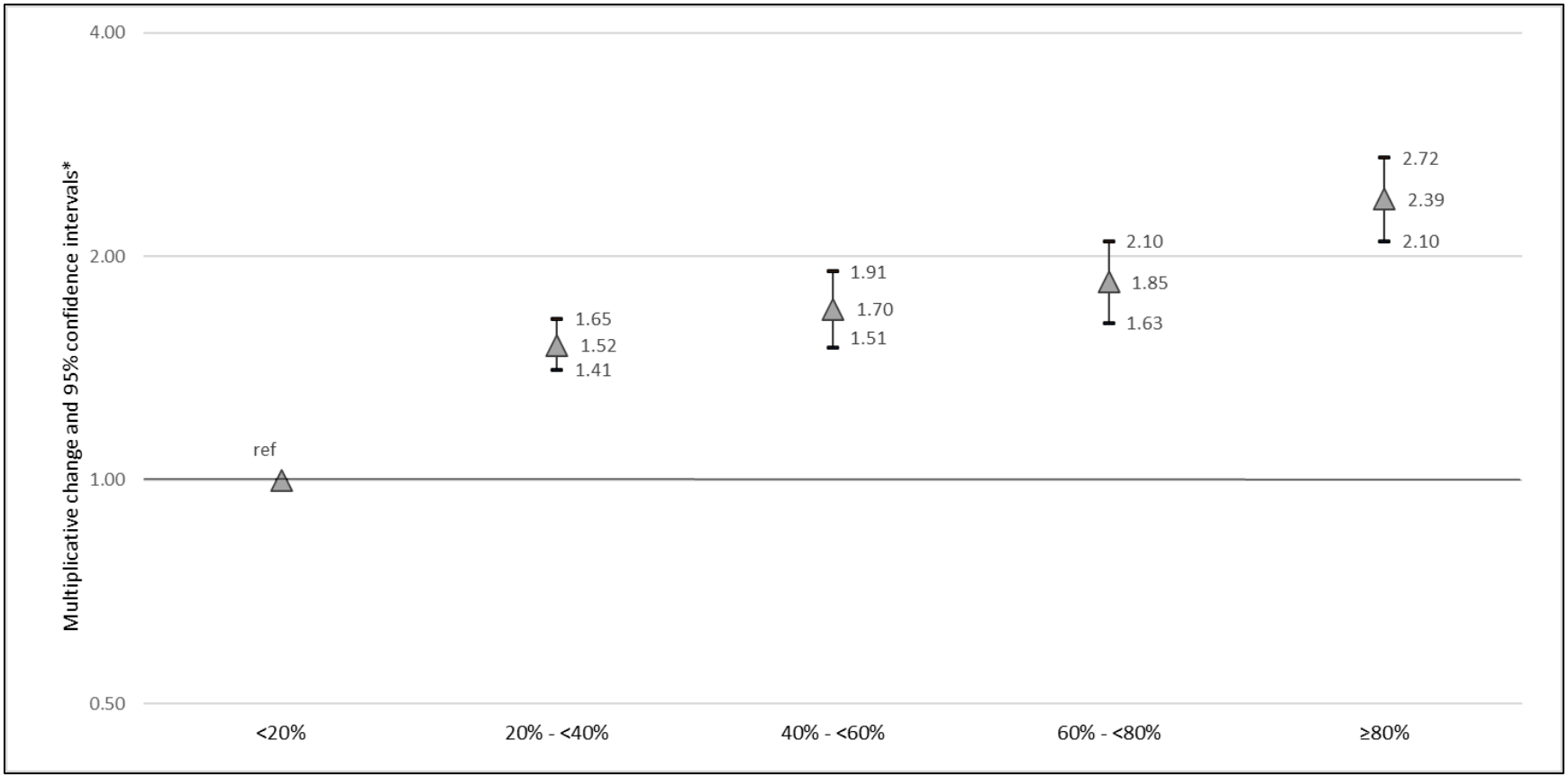
TICS denotes the Telephone Interview for Cognitive Status. The global score is a composite of all 8 cognitive tests. The memory score is a composite of 4 tests, the immediate and delayed recalls of the TICS 10-word list and the East Boston Memory Test. The executive function score is a composite of the Digit Span Backwards Test and the Oral Trail Making Test part B. Attention was assessed with the Oral Trail Making Test part A. Language was assessed through the Animal Naming Test for verbal fluency.



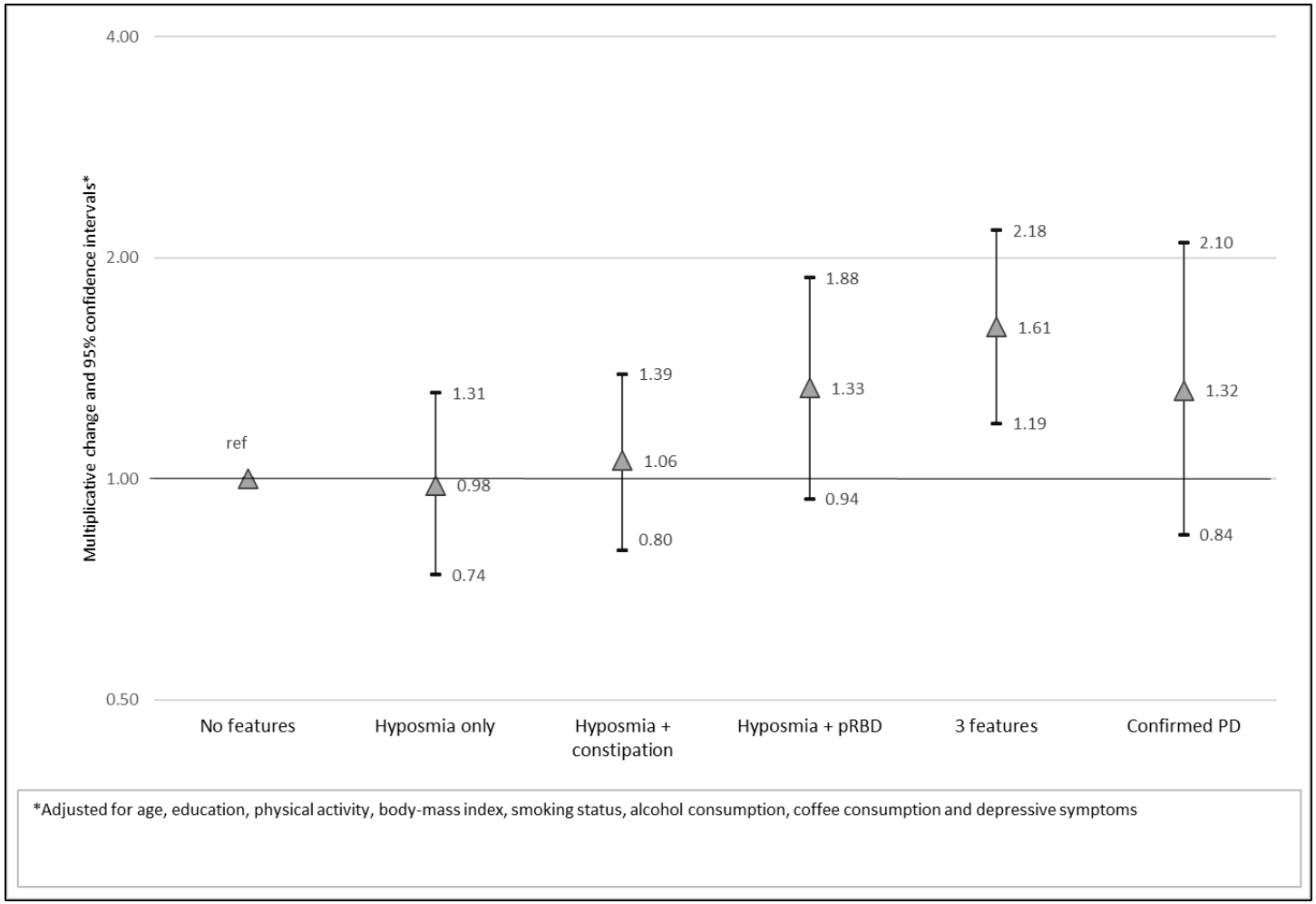
Supplemental Figure 2.1 Flow chart of the study design.



Supplemental Figure 2.2. Multivariable-adjusted multiplicative change in the mean subjective cognitive decline score and 95% confidence intervals according to the presence of prodromal features and diagnosed Parkinson's disease (PD).



Supplemental figure 2.3. Age- and education-adjusted multiplicative change in the mean subjective cognitive decline score and 95% confidence intervals according to the probability of prodromal Parkinson's disease.



Supplemental figure 2.4. Multivariable-adjusted multiplicative change in the mean subjective cognitive decline score and 95% confidence intervals according to the presence of prodromal features and diagnosed Parkinson's disease (PD) in the subset population.

Appendix 3: Supplemental Tables for Chapter 3

Supplemental table 3.1. Multivariable adjusted hazard ratios of Parkinson's disease and 95% confidence intervals according to different lagged exposure periods of the cumulative average intake of folate, vitamin B6 and vitamin B12, 1984-86 to 2016

	Women		Men		Pooled			
Folate	8-year lag	20-year lag	8-year lag	20-year lag	8-year lag	12-year lag	16-year lag	20-year lag
Total								
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	1.15 (0.88, 1.51)	1.23 (0.87, 1.75)	0.86 (0.64, 1.14)	0.99 (0.66, 1.47)	1.00 (0.82, 1.22)	0.97 (0.79, 1.20)	1.12 (0.89, 1.41)	1.12 (0.86, 1.46)
Quintile 3	1.15 (0.88, 1.51)	1.02 (0.71, 1.48)	0.96 (0.72, 1.27)	0.99 (0.66, 1.49)	1.05 (0.87, 1.28)	1.00 (0.81, 1.24)	0.99 (0.78, 1.26)	1.02 (0.77, 1.33)
Quintile 4	0.87 (0.65, 1.16)	0.87 (0.59, 1.27)	1.00 (0.76, 1.33)	0.98 (0.65, 1.47)	0.94 (0.77,1.14)	1.00 (0.81, 1.24)	1.14 (0.90, 1.44)	0.91 (0.69, 1.21)
Quintile 5	1.04 (0.78, 1.36)	0.96 (0.66, 1.40)	0.94 (0.71, 1.25)	0.97 (0.64, 1.48)	0.99 (0.81, 1.21)	0.95 (0.76, 1.18)	0.91 (0.71, 1.16)	0.97 (0.73, 1.28)
P-trend	*0.44*	*0.26*	*0.90*	*0.88*	*0.71*	*0.77*	*0.27*	*0.38*
P-het					*0.49*	*0.36*	*0.25*	*0.47*
Dietary								
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	1.07 (0.81, 1.41)	0.74 (0.52, 1.06)	1.00 (0.76, 1.34)	0.98 (0.66, 1.47)	1.04 (0.85, 1.27)	1.04 (0.84, 1.29)	1.12 (0.88, 1.41)	0.84 (0.64, 1.10)
Quintile 3	1.04 (0.79, 1.38)	0.98 (0.69, 1.38)	1.00 (0.74, 1.34)	1.05 (0.70, 1.57)	1.02 (0.83, 1.25)	0.98 (0.78, 1.22)	1.21 (0.96, 1.53)	1.01 (0.77, 1.31)
Quintile 4	1.01 (0.75, 1.35)	0.80 (0.55, 1.16)	1.11 (0.82, 1.49)	0.83 (0.54, 1.27)	1.06 (0.86, 1.30)	0.97 (0.77, 1.21)	0.91 (0.71, 1.18)	0.81 (0.61, 1.08)
Quintile 5	1.07 (0.79, 1.44)	0.68 (0.46, 1.00)	1.10 (0.81, 1.50)	0.88 (0.57, 1.37)	1.08 (0.87, 1.35)	1.10 (0.87, 1.38)	1.06 (0.82, 1.37)	0.76 (0.57, 1.02)
P-trend	*0.81*	*0.08*	*0.41*	*0.39*	*0.42*	*0.38*	*0.88*	*0.07*
P-het					*0.76*	*0.29*	*0.20*	*0.44*
Supplemental								
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	0.95 (0.73, 1.25)	0.81 (0.53, 1.24)	1.00 (0.75, 1.35)	0.66 (0.37, 1.18)	0.98 (0.80, 1.19)	1.02 (0.81, 1.29)	0.88 (0.67, 1.14)	0.75 (0.54, 1.06)
Quintile 3	1.00 (0.76, 1.31)	1.09 (0.72, 1.64)	0.93 (0.70, 1.22)	0.75 (0.47, 1.19)	0.96 (0.80, 1.17)	1.18 (0.95, 1.46)	0.88 (0.69, 1.14)	0.92 (0.68, 1.25)
Quintile 4	0.85 (0.65, 1.12)	0.80 (0.53, 1.19)	0.89 (0.68, 1.15)	0.82 (0.55, 1.23)	0.87 (0.72, 1.05)	0.99 (0.81, 1.23)	0.85 (0.68, 1.08)	0.81 (0.61, 1.08)
Quintile 5	0.90 (0.69, 1.17)	0.90 (0.62, 1.30)	0.92 (0.71, 1.18)	0.79 (0.55, 1.14)	0.91 (0.76, 1.09)	0.94 (0.77, 1.15)	0.85 (0.68, 1.06)	0.84 (0.65, 1.09)
P-trend	*0.31*	*0.51*	*0.53*	*0.48*	*0.25*	*0.29*	*0.42*	*0.34*
P-het					*0.70*	*0.89*	*0.97*	*0.98*
Vitamin B6	8-year lag	20-year lag	8-year lag	20-year lag	8-year lag	12-year lag	16-year lag	20-year lag
Total								
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	1.12 (0.86, 1.45)	0.83 (0.60, 1.15)	1.21 (0.92, 1.59)	0.95 (0.64, 1.41)	1.16 (0.96, 1.40)	1.14 (0.92, 1.40)	1.13 (0.90, 1.40)	0.88 (0.68, 1.13)
Quintile 3	1.00 (0.76, 1.30)	0.61 (0.42, 0.88)	1.06 (0.80, 1.41)	1.13 (0.77, 1.65)	1.03 (0.85, 1.25)	1.15 (0.94, 1.42)	1.03 (0.82, 1.30)	0.82 (0.63, 1.06)
Quintile 4	0.88 (0.66, 1.15)	0.80 (0.57, 1.12)	1.08 (0.82, 1.43)	1.15 (0.79, 1.68)	0.97 (0.80, 1.18)	1.02 (0.82, 1.26)	0.94 (0.75, 1.18)	0.94 (0.73, 1.21)
Quintile 5	1.05 (0.80, 1.38)	0.78 (0.55, 1.09)	1.01 (0.76, 1.34)	0.70 (0.46, 1.08)	1.03 (0.85, 1.25)	1.05 (0.84, 1.29)	0.95 (0.76, 1.20)	0.75 (0.57, 0.97)
P-trend	*0.56*	*0.64*	*0.57*	*0.03*	*0.92*	*0.51*	*0.40*	*0.20*
P-het					*0.42*	*0.18*	*0.25*	*0.06*
Dietary								
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	1.08 (0.82, 1.41)	0.88 (0.63, 1.24)	0.96 (0.71, 1.28)	1.12 (0.74, 1.67)	1.02 (0.84, 1.24)	0.96 (0.78, 1.19)	0.91 (0.72, 1.15)	0.97 (0.75, 1.26)
Quintile 3	1.07 (0.81, 1.41)	0.89 (0.63, 1.25)	1.09 (0.82, 1.44)	1.26 (0.84, 1.88)	1.08 (0.89, 1.31)	1.09 (0.88, 1.34)	1.11 (0.89, 1.39)	1.03 (0.79, 1.33)
Quintile 4	1.06 (0.80, 1.41)	0.89 (0.62, 1.26)	1.00 (0.75, 1.33)	0.92 (0.59, 1.41)	1.03 (0.84, 1.26)	0.97 (0.78, 1.20)	1.03 (0.82, 1.30)	0.90 (0.68, 1.18)
Quintile 5	0.97 (0.73, 1.30)	0.64 (0.44, 0.93)	1.09 (0.82, 1.46)	1.20 (0.79, 1.84)	1.03 (0.84, 1.27)	1.00 (0.80, 1.25)	0.82 (0.64, 1.05)	0.85 (0.64, 1.12)
P-trend	*0.70*	*0.02*	*0.55*	*0.72*	*0.80*	*0.82*	*0.23*	*0.24*
P-het					*0.51*	*0.19*	*0.07*	*0.05*
Supplemental								
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	1.05 (0.81, 1.37)	1.07 (0.73, 1.57)	1.10 (0.83, 1.46)	0.93 (0.58, 1.48)	1.08 (0.89, 1.30)	1.10 (0.88, 1.37)	1.02 (0.80, 1.30)	1.01 (0.75, 1.36)
Quintile 3	0.93 (0.71, 1.21)	1.20 (0.81, 1.76)	1.00 (0.76, 1.31)	1.00 (0.65, 1.52)	0.96 (0.80, 1.16)	1.11 (0.90, 1.37)	1.09 (0.86, 1.38)	1.10 (0.83, 1.47)
Quintile 4	0.83 (0.63, 1.08)	0.89 (0.62, 1.29)	0.98 (0.76, 1.27)	1.03 (0.71, 1.49)	0.90 (0.75, 1.080	1.00 (0.82, 1.23)	0.90 (0.72, 1.13)	0.96 (0.74, 1.25)

Supplemental table 3.1 (continued)

Quintile 5	1.02 (0.79, 1.32)	1.07 (0.75, 1.53)	0.93 (0.71, 1.20)	0.81 (0.55, 1.19)	0.97 (0.81, 1.17)	1.01 (0.83, 1.24)	0.93 (0.75, 1.16)	0.94 (0.72, 1.22)
P-trend	*0.41*	*0.86*	*0.58*	*0.16*	*0.76*	*0.53*	*0.49*	*0.48*
P-het					*0.35*	*0.26*	*0.14*	*0.22*
Vitamin B12	8-year lag	20-year lag	8-year lag	20-year lag	8-year lag	12-year lag	16-year lag	20-year lag
Total								
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	1.00 (0.78, 1.28)	0.76 (0.55, 1.07)	0.95 (0.73, 1.24)	1.32 (0.91, 1.91)	0.98 (0.82, 1.17)	1.17 (0.97, 1.42)	1.14 (0.92, 1.40)	0.98 (0.76, 1.25)
Quintile 3	0.82 (0.63, 1.06)	0.89 (0.65, 1.22)	0.93 (0.72, 1.21)	1.36 (0.94, 1.95)	0.87 (0.73, 1.05)	0.98 (0.81, 1.20)	0.96 (0.78, 1.19)	1.07 (0.84, 1.36)
Quintile 4	0.88 (0.68, 1.13)	0.85 (0.62, 1.16)	0.92 (0.71, 1.19)	0.89 (0.59, 1.34)	0.90 (0.75, 1.07)	0.88 (0.71, 1.08)	0.94 (0.76, 1.18)	0.87 (0.67, 1.11)
Quintile 5	0.85 (0.66, 1.10)	0.70 (0.50, 0.99)	0.94 (0.73, 1.21)	0.88 (0.59, 1.32)	0.90 (0.75, 1.07)	1.03 (0.85, 1.25)	0.88 (0.70, 1.09)	0.77 (0.60, 1.01)
P-trend	*0.44*	*0.14*	*0.67*	*0.08*	*0.42*	*0.49*	*0.07*	*0.02*
P-het					*0.72*	*0.82*	*0.95*	*0.86*
Dietary								
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	1.03 (0.80, 1.32)	0.97 (0.70, 1.36)	0.99 (0.76, 1.280	1.01 (0.69, 1.48)	1.01 (0.84, 1.21)	0.99 (0.81, 1.21)	1.02 (0.82, 1.27)	0.99 (0.77, 1.27)
Quintile 3	0.91 (0.70, 1.18)	0.91 (0.65, 1.27)	0.85 (0.65, 1.100	1.12 (0.77, 1.64)	0.88 (0.73, 1.06)	1.02 (0.84, 1.24)	1.08 (0.87, 1.34)	1.00 (0.78, 1.29)
Quintile 4	1.14 (0.89, 1.46)	1.02 (0.73, 1.41)	0.93 (0.72, 1.21)	1.01 (0.68, 1.48)	1.04 (0.87, 1.24)	1.05 (0.86, 1.27)	1.08 (0.87, 1.33)	1.01 (0.79, 1.30)
Quintile 5	0.79 (0.60, 1.04)	0.82 (0.58, 1.16)	0.93 (0.72, 1.20)	0.82 (0.54, 1.22)	0.86 (0.71, 1.03)	0.79 (0.64, 0.98)	0.83 (0.66, 1.04)	0.82 (0.63, 1.06)
P-trend	*0.11*	*0.37*	*0.86*	*0.20*	*0.24*	*0.05*	*0.11*	*0.12*
P-het					*0.26*	*0.30*	*0.90*	*0.73*
Supplemental								
Quintile 1	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.	Ref.
Quintile 2	1.22 (0.95, 1.57)	0.87 (0.62, 1.21)	1.12 (0.86, 1.46)	0.89 (0.59, 1.34)	1.17 (0.98, 1.41)	0.89 (0.72, 1.09)	0.91 (0.73, 1.13)	0.88 (0.68, 1.14)
Quintile 3	0.82 (0.63,1.08)	0.65 (0.46, 0.94)	1.03 (0.79, 1.35)	0.96 (0.66, 1.40)	0.93 (0.76, 1.12)	0.96 (0.78, 1.17)	0.91 (0.73, 1.14)	0.79 (0.61, 1.02)
Quintile 4	0.96 (0.74, 1.24)	0.62 (0.44, 0.89)	0.93 (0.71, 1.21)	0.92 (0.64, 1.32)	0.94 (0.78, 1.13)	0.87 (0.71, 1.060	0.83 (0.66, 1.03)	0.75 (0.58, 0.97)
Quintile 5	0.92 (0.71, 1.19)	0.80 (0.58, 1.10)	0.97 (0.75, 1.25)	0.78 (0.54, 1.13)	0.94 (0.79, 1.13)	0.99 (0.82, 1.20)	0.93 (0.76, 1.14)	0.79 (0.62, 1.01)
P-trend	*0.86*	*0.42*	*0.75*	*0.21*	*0.72*	*0.50*	*0.78*	*0.14*
P-het					*0.96*	*0.67*	*0.83*	*0.74*

All estimates are adjusted for age in months, calendar time (2-year periods), total energy intake (quintiles) body mass index (<25, 25-30, 30+), smoking (never, ≤4, 4-24 and ≥25 pack-years), alcohol (0 to <5, 5 to <10, 10 to <15, 15 to <30 and ≥30 g/day) and caffeine intake (quintiles), physical activity (quintiles), hormone use (only women; premenopausal, no use, current use and past use), flavonoids intake (quintiles), dairy intake (quintiles) and Mediterranean diet score (quintiles).

Supplemental table 3.2. Multivariable adjusted hazard ratios of Parkinson's disease and 95% confidence intervals according to recent intake of folate, vitamin B6 and vitamin B12, 1984-86 to 2016

	Women	Men	Pooled
Folate			
Total			
Quintile 1	Ref.	Ref.	Ref.
Quintile 2	1.04 (0.79, 1.35)	0.99 (0.75, 1.28)	1.01 (0.83, 1.21)
Quintile 3	1.16 (0.89, 1.50)	0.98 (0.75, 1.27)	1.07 (0.89, 1.28)
Quintile 4	1.04 (0.80, 1.35)	1.14 (0.88, 1.48)	1.09 (0.91, 1.31)
Quintile 5	1.06 (0.82, 1.39)	0.93 (0.71, 1.22)	1.00 (0.83, 1.20)
P-trend	*0.64*	*0.81*	*0.90*
P-het			*0.61*
Dietary			
Quintile 1	Ref.	Ref.	Ref.
Quintile 2	0.82 (0.64, 1.07)	1.01 (0.77, 1.31)	0.91 (0.76, 1.10)
Quintile 3	0.92 (0.71, 1.19)	1.11 (0.86, 1.45)	1.01 (0.84, 1.21)
Quintile 4	0.82 (0.63, 1.07)	0.91 (0.69, 1.20)	0.86 (0.71, 1.04)
Quintile 5	0.88 (0.67, 1.14)	1.03 (0.78, 1.36)	0.95 (0.78, 1.15)
P-trend	*0.43*	*0.94*	*0.46*
P-het			*0.99*
Supplemental			
Quintile 1	Ref.	Ref.	Ref.
Quintile 2	1.07 (0.80, 1.42)	1.05 (0.77, 1.44)	1.06 (0.86, 1.31)
Quintile 3	1.16 (0.87, 1.55)	0.89 (0.65, 1.20)	1.02 (0.83, 1.26)
Quintile 4	0.99 (0.76, 1.28)	1.21 (0.95, 1.54)	1.10 (0.92, 1.31)
Quintile 5	1.16 (0.90, 1.49)	1.02 (0.80, 1.30)	1.09 (0.91, 1.29)
P-trend	*0.14*	*0.95*	*0.31*
P-het			*0.30*
Vitamin B6			
Total			
Quintile 1	Ref.	Ref.	Ref.
Quintile 2	1.41 (1.08, 1.85)	1.05 (0.80, 1.36)	1.21 (1.00, 1.46)
Quintile 3	1.37 (1.05, 1.79)	1.05 (0.81, 1.37)	1.20 (0.99, 1.44)
Quintile 4	1.20 (0.91, 1.57)	1.01 (0.77, 1.31)	1.09 (0.90, 1.32)
Quintile 5	1.32 (1.00, 1.73)	1.05 (0.81, 1.36)	1.17 (0.97, 1.41)
P-trend	*0.34*	*0.49*	*0.84*
P-het			*0.25*
Dietary			
Quintile 1	Ref.	Ref.	Ref.
Quintile 2	0.94 (0.72, 1.21)	0.96 (0.74, 1.26)	0.95 (0.79, 1.14)
Quintile 3	0.96 (0.74, 1.24)	1.01 (0.78, 1.32)	0.98 (0.82, 1.18)
Quintile 4	1.05 (0.81, 1.36)	1.00 (0.77, 1.31)	1.03 (0.86, 1.24)
Quintile 5	0.86 (0.66, 1.12)	1.05 (0.80, 1.36)	0.95 (0.78, 1.14)
P-trend	*0.42*	*0.60*	*0.90*
P-het			*0.34*
Supplemental			
Quintile 1	Ref.	Ref.	Ref.
Quintile 2	1.23 (0.92, 1.62)	1.27 (0.94, 1.71)	1.25 (1.02, 1.53)
Quintile 3	1.11 (0.85, 1.46)	1.34 (1.02, 1.75)	1.22 (1.01, 1.48)
Quintile 4	1.22 (0.95, 1.58)	1.07 (0.83, 1.38)	1.14 (0.95, 1.37)
Quintile 5	1.23 (0.95, 1.59)	1.13 (0.88, 1.45)	1.18 (0.98, 1.41)
P-trend	*0.28*	*0.36*	*0.89*
P-het			*0.16*
Vitamin B12			

Supplemental table 3.2 (continued)

Total			
Quintile 1	Ref.	Ref.	Ref.
Quintile 2	1.14 (0.89, 1.48)	0.96 (0.74, 1.23)	1.05 (0.87, 1.25)
Quintile 3	1.12 (0.86, 1.44)	0.86 (0.66, 1.11)	0.98 (0.82, 1.17)
Quintile 4	1.15 (0.89, 1.48)	0.96 (0.75, 1.23)	1.05 (0.88, 1.25)
Quintile 5	1.03 (0.79, 1.33)	0.94 (0.74,1.20)	0.98 (0.82, 1.17)
P-trend	*0.20*	*0.68*	*0.22*
P-het			*0.58*
Dietary			
Quintile 1	Ref.	Ref.	Ref.
Quintile 2	1.24 (0.96, 1.59)	1.00 (0.78, 1.28)	1.11 (0.93, 1.33)
Quintile 3	1.10 (0.85, 1.43)	0.84 (0.65, 1.09)	0.96 (0.80, 1.16)
Quintile 4	1.05 (0.81, 1.36)	0.87 (0.67, 1.13)	0.95 (0.79, 1.15)
Quintile 5	1.10 (0.85, 1.43)	0.94 (0.74, 1.21)	1.01 (0.85, 1.21)
P-trend	*0.78*	*0.72*	*0.65*
P-het			*0.99*
Supplemental			
Quintile 1	Ref.	Ref.	Ref.
Quintile 2	1.20 (0.91, 1.59)	1.35 (1.02, 1.79)	1.27 (1.04, 1.55)
Quintile 3	1.08 (0.83, 1.40)	1.19 (0.92, 1.54)	1.13 (0.94, 1.36)
Quintile 4	1.11 (0.85, 1.43)	1.11 (0.86, 1.42)	1.11 (0.92, 1.33)
Quintile 5	1.15 (0.90, 1.48)	1.07 (0.83, 1.37)	1.11 (0.93, 1.32)
P-trend	*0.25*	*0.69*	*0.26*
P-het			*0.64*

All estimates are adjusted for age in months, calendar time (2-year periods), total energy intake (quintiles) body mass index (<25, 25-30, 30+), smoking (never, ≤4, 4-24 and ≥25 pack-years), alcohol (0 to <5, 5 to <10, 10 to <15, 15 to <30 and ≥30 g/day) and caffeine intake (quintiles), physical activity (quintiles), hormone use (only women; premenopausal, no use, current use and past use), flavonoids intake (quintiles), dairy intake (quintiles) and Mediterranean diet score (quintiles).

Supplemental table 3.3. Multivariable adjusted hazard ratios of Parkinson's disease and 95% confidence intervals according to baseline (1984-86) intake levels of folate, vitamin B6 and vitamin B12 at different periods

	Baseline to 1998	1998 to 2006	2006 to 2016
No. of cases			
Total folate			
Quintile 1	Ref.	Ref.	Ref.
Quintile 2	1.29 (0.90, 1.84)	1.08 (0.78, 1.48)	1.06 (0.80, 1.41)
Quintile 3	1.47 (1.03, 2.10)	0.95 (0.68, 1.33)	1.12 (0.84, 1.50)
Quintile 4	1.38 (0.96, 1.980	0.94 (0.67, 1.31)	0.82 (0.60, 1.11)
Quintile 5	1.22 (0.85, 1.75)	0.95 (0.68, 1.32)	1.01 (0.75, 1.35)
P-trend	*0.96*	*0.59*	*0.62*
P-het	*0.70*	*0.53*	*0.30*
Total B6			
Quintile 1	Ref.	Ref.	Ref.
Quintile 2	1.50 (1.06, 2.11)	0.88 (0.64, 1.20)	0.89 (0.68, 1.17)
Quintile 3	1.19 (0.83, 1.70)	0.97 (0.71, 1.33)	0.89 (0.68, 1.16)
Quintile 4	1.32 (0.94, 1.86)	0.81 (0.59, 1.12)	0.85 (0.65, 1.12)
Quintile 5	1.16 (0.82, 1.65)	0.97 (0.72, 1.32)	0.81 (0.61, 1.06)
P-trend	*0.71*	*0.79*	*0.25*
P-het	*0.93*	*0.96*	*0.25*
Total B12			
Quintile 1	Ref.	Ref.	Ref.
Quintile 2	1.06 (0.77, 1.46)	0.95 (0.70, 1.29)	0.97 (0.75, 1.26)
Quintile 3	1.28 (0.95, 1.71)	0.91 (0.67, 1.22)	1.05 (0.82, 1.35)
Quintile 4	0.89 (0.65, 1.21)	1.08 (0.81, 1.43)	0.97 (0.75, 1.26)
Quintile 5	0.85 (0.62, 1.18)	0.88 (0.65, 1.18)	0.70 (0.53, 0.92)
P-trend	*0.15*	*0.74*	*0.01*
P-het	*0.45*	*0.94*	*0.86*

All estimates are adjusted for age in months, calendar time (2-year periods), total energy intake (quintiles) body mass index (<25, 25-30, 30+), smoking (never, ≤4, 4-24 and ≥25 pack-years), alcohol (0 to <5, 5 to <10, 10 to <15, 15 to <30 and ≥30 g/day) and caffeine intake (quintiles), physical activity (quintiles), hormone use (only women; premenopausal, no use, current use and past use), flavonoids intake (quintiles), dairy intake (quintiles) and Mediterranean diet score (quintiles).

Supplemental table 3.4. Multivariable adjusted hazard ratios of Parkinson's disease and 95% confidence intervals according to baseline dietary intake of vitamin B12 among individuals with no B12 intake from supplements at baseline

	Women (n=23,871)		Men (n=15,385)		Pooled
	Median (mcg/day)	HR (95% CI)	Median (mcg/day)	HR (95% CI)	
Dietary B12					
Quintile 1	3.2	Ref.	4.4	Ref.	Ref.
Quintile 2	4.4	0.99 (0.64, 1.55)	6.2	1.02 (0.66, 1.57)	1.01 (0.74, 1.37)
Quintile 3	5.7	1.21 (0.78,1.88)	7.7	1.24 (0.80, 1.92)	1.22 (0.90, 1.67)
Quintile 4	8.6	1.14 (0.73, 1.78)	9.6	1.28 (0.83, 1.97)	1.21 (0.88, 1.65)
Quintile 5	17.4	1.08 (0.68, 1.72)	18.3	1.11 (0.71, 1.72)	1.10 (0.80, 1.51)
P-trend		*0.84*		*0.72*	*0.69*
P-het					*0.91*

All estimates are adjusted for age in months, calendar time (2-year periods), total energy intake (quintiles) body mass index (<25, 25-30, 30+), smoking (never, ≤4, 4-24 and ≥25 pack-years), alcohol (0 to <5, 5 to <10, 10 to <15, 15 to <30 and ≥30 g/day) and caffeine intake (quintiles), physical activity (quintiles), hormone use (only women; premenopausal, no use, current use and past use), flavonoids intake (quintiles), dairy intake (quintiles) and Mediterranean diet score (quintiles).

Supplemental table 3.5. Multivariable adjusted hazard ratios of Parkinson's disease and 95% confidence intervals according to baseline supplemental intake of vitamin B12 among individuals in the 2 lowest quintiles of dietary B12 intake at baseline

	Women (n=32,476)		Men (n=19,592)		Pooled
	Median (mcg/day)	HR (95% CI)	Median (mcg/day)	HR (95% CI)	
Supplemental B12					
Quintile 1	0	Ref.	0	Ref.	Ref.
Quintile 2	0.2	0.91 (0.60, 1.39)	0.2	0.83 (0.54, 1.28)	0.87 (0.65, 1.18)
Quintile 3	0.4	0.81 (0.54, 1.22)	0.6	1.33 (0.93, 1.89)	1.07 (0.82, 1.40)
Quintile 4	2.3	0.84 (0.57, 1.23)	3.2	0.87 (0.60, 1.26)	0.85 (0.65, 1.11)
Quintile 5	7.2	1.05 (0.74, 1.47)	8.9	0.90 (0.63, 1.28)	0.97 (0.76, 1.24)
P-trend		*0.51*		*0.33*	*0.75*
P-het					*0.26*

All estimates are adjusted for age in months, calendar time (2-year periods), total energy intake (quintiles) body mass index (<25, 25-30, 30+), smoking (never, ≤4, 4-24 and ≥25 pack-years), alcohol (0 to <5, 5 to <10, 10 to <15, 15 to <30 and ≥30 g/day) and caffeine intake (quintiles), physical activity (quintiles), hormone use (only women; premenopausal, no use, current use and past use), flavonoids intake (quintiles), dairy intake (quintiles) and Mediterranean diet score (quintiles).

Supplemental table 3.6. Multivariable adjusted hazard ratios of Parkinson's disease and 95% confidence intervals according to use of multivitamins and B complex at baseline

	Women		Men		Pooled	
	n (%)	HR (95% CI)	n (%)	HR (95% CI)	HR (95% CI)	*P-het*
Use of multivitamins*						
Never	37,084 (46.0)	Ref.	18,036 (40.5)	Ref.	Ref.	
Past	13,827 (17.1)	0.91 (0.74, 1.13)	9,403 (21.1)	1.03 (0.84, 1.26)	0.97 (0.84, 1.13)	*0.44*
≤ 5 pills/wk	9,660 (12.0)	0.80 (0.61, 1.03)	5,086 (11.4)	0.67 (0.50, 0.91)	0.74 (0.61, 0.90)	*0.41*
6-9 pills/wk	17,318 (21.5)	0.90 (0.73, 1.09)	9,326 (20.1)	1.04 (0.85, 1.27)	0.96 (0.83, 1.11)	*0.32*
≥10 pills/wk	2,792 (3.5)	0.72 (0.46, 1.13)	2,671 (6.0)	1.04 (0.76, 1.43)	0.92 (0.71, 1.19)	*0.19*
Use of B complex						
No	73,019 (90.2)	Ref.	43,870 (89.8)	Ref.	Ref.	
Yes	7,946 (9.8)	1.08 (0.85, 1.39)	4,967 (10.2)	0.91 (0.71, 1.18)	1.00 (0.84, 1.19)	*0.34*
Combined use*						
None	36,288 (45.0)	Ref.	17,756 (39.9)	Ref.	Ref.	
B complex only	796 (1.0)	0.97 (0.45, 2.05)	280 (0.6)	1.82 (0.84, 3.95)	1.32 (0.77, 2.26)	*0.25*
Multivitamins only	36,514 (45.3)	0.84 (0.72, 0.99)	22,648 (50.9)	1.00 (0.85, 1.17)	0.91 (0.82, 1.02)	*0.15*
Both	7,083 (8.8)	1.00 (0.77, 1.31)	3,838 (8.6)	0.86 (0.63, 1.16)	0.94 (0.76, 1.15)	*0.45*

All estimates are adjusted for age in months, calendar time (2-year periods), total energy intake (quintiles) body mass index (<25, 25-30, 30+), smoking (never, ≤4, 4-24 and ≥25 pack-years), alcohol (0 to <5, 5 to <10, 10 to <15, 15 to <30 and ≥30 g/day) and caffeine intake (quintiles), physical activity (quintiles), hormone use (only women; premenopausal, no use, current use and past use), flavonoids intake (quintiles), dairy intake (quintiles) and Mediterranean diet score (quintiles). *284 women and 4,315 men were excluded from analyses due to missing values on multivitamin use

Supplemental table 3.7. Multivariable adjusted hazard ratios of Parkinson's disease and 95% confidence interval according to deciles of baseline intake of folate, vitamin B6 and vitamin B12, 1984-86 to 2016

	Women			Men			Pooled
Total folate	n	Median, mcg/day	HR and 95% CI	n	Median, mcg/day	HR and 95% CI	HR and 95% CI
Decile 1	8,274	163	0.79 (0.52, 1.18)	4,915	216	1.13 (0.78, 1.62)	0.96 (0.73, 1.26)
Decile 2	8,065	203	1.00 (0.59, 1.43)	4.974	267	1.09 (0.77, 1.53)	1.04 (0.81, 1.34)
Decile 3	8,196	231	1.10 (0.78, 1.54)	4,765	302	1.09 (0.78, 1.54)	1.10 (0.86, 1.39)
Decile 4	8,139	258	1.12 (0.80, 1.56)	4,960	334	0.99 (0.70, 1.39)	1.05 (0.83, 1.34)
Decile 5	7,999	286	Ref.	4,836	369	Ref.	Ref.
Decile 6	8,043	322	1.00 (0.72, 1.40)	4,889	410	1.29 (0.93, 1.77)	1.14 (0.90, 1.44)
Decile 7	7,971	375	0.92 (0.66, 1.29)	4,859	471	1.14 (0.82, 1.58)	1.03 (0.81, 1.30)
Decile 8	8,102	480	0.93 (0.67, 1.31)	4,875	582	0.97 (0.69, 1.37)	0.95 (0.75, 1.21)
Decile 9	8,086	629	0.87 (0.61, 1.22)	4,888	744	1.37 (0.99, 1.88)	1.11 (0.88, 1.40)
Decile 10	8,090	825	0.83 (0.58, 1.18)	4,876	1,041	0.93 (0.66, 1.32)	0.88 (0.69, 1.13)
p-het							
Total vitamin B6	n	Median, mcg/day	HR and 95% CI	n	Median, mcg/day	HR and 95% CI	HR and 95% CI
Decile 1	9,272	1.2	0.88 (0.59, 1.29)	6,392	1.6	1.11 (0.80, 1.54)	1.01 (0.78, 1.30)
Decile 2	8,714	1.5	1.02 (0.71, 1.47)	4,354	1.9	0.91 (0.64, 1.31)	0.96 (0.75, 1.25)
Decile 3	9,315	1.6	0.90 (0.63, 1.29)	4,631	2.0	1.16 (0.83, 1.61)	1.03 (0.81, 1.32)
Decile 4	8,267	1.8	0.90 (0.63, 1.29)	4,179	2.2	1.08 (0.77, 1.52)	0.99 (0.78, 1.27)
Decile 5	5,947	2.0	Ref.	5,006	2.5	Ref.	Ref.
Decile 6	7,739	2.3	0.99 (0.70, 1.41)	4,789	2.9	1.02 (0.74, 1.42)	1.01 (0.79, 1.28)
Decile 7	8,174	3.2	0.82 (0.57, 1.19)	5,103	3.6	1.10 (0.80, 1.51)	0.97 (0.76, 1.23)
Decile 8	7,509	4.1	0.74 (0.51, 1.08)	4,700	4.8	1.28 (0.93, 1.75)	1.02 (0.80, 1.30)
Decile 9	7,966	6.4	0.96 (0.67, 1.37)	4,810	7.2	1.07 (0.77, 1.48)	1.02 (0.80, 1.30)
Decile 10	8,062	57.4	0.87 (0.60, 1.26)	4,873	34.9	0.90 (0.64, 1.26)	0.89 (0.69, 1.14)
p-het							
Total vitamin B12	n	Median, mcg/day	HR and 95% CI	n	Median, mcg/day	HR and 95% CI	HR and 95% CI
Decile 1	14,197	4	0.89 (0.64, 1.23)	6,873	5	0.83 (0.60, 1.15)	0.86 (0.68, 1.08)
Decile 2	9,125	5	1.01 (0.71, 1.42)	4,559	6	1.05 (0.75, 1.48)	1.03 (0.81, 1.31)
Decile 3	7,384	6	0.95 (0.66, 1.37)	4,780	7	1.04 (0.75, 1.45)	1.00 (0.78, 1.28)
Decile 4	5,191	7	0.96 (0.65, 1.37)	4,346	8	0.82 (0.57, 1.17)	0.88 (0.67, 1.15)
Decile 5	6,629	8	Ref.	3,887	9	Ref.	Ref.
Decile 6	6,934	11	0.96 (0.67, 1.37)	6,288	10	1.02 (0.75, 1.40)	0.99 (0.78, 1.26)
Decile 7	7,868	12	1.06 (0.75, 1.49)	4,365	12	0.91 (0.65, 1.29)	0.98 (0.77, 1.25)
Decile 8	9,518	15	0.96 (0.68, 1.34)	4,403	15	0.78 (0.55, 1.11)	0.87 (0.68, 1.11)
Decile 9	7,038	18	0.61 (0.40, 0.91)	4,761	19	0.82 (0.58, 1.17)	0.72 (0.56, 0.94)
Decile 10	7,081	25	0.71 (0.48, 1.05)	4,575	28	0.84 (0.59, 1.19)	0.78 (0.60, 1.01)
p-het							

Supplemental table 3.7 (continued)

All estimates are adjusted for age in months, calendar time (2-year periods), total energy intake (quintiles) body mass index (<25, 25-30, 30+), smoking (never, ≤4, 4-24 and ≥25 pack-years), alcohol and caffeine intake (quintiles), physical activity (quintiles), hormone use (only women; premenopausal, no use, current use and past use), flavonoids intake (quintiles), dairy intake (quintiles) and Mediterranean diet score (quintiles).