RESEARCH ARTICLE

Dietary Antioxidants and Risk of Parkinson's Disease in the Singapore Chinese Health Study

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ABSTRACT: Background: Despite experimental evidence implicating oxidative stress in the pathogenesis of PD, epidemiological studies have provided inconsistent associations between dietary antioxidants and risk of developing PD. Furthermore, no study has been done in any Asian population.

Objectives: We examined the associations for intake levels of dietary carotenoids (α-carotene, β-carotene, lycopene, β-cryptoxanthin, and lutein) and vitamins (vitamin A, C and E) and the risk of developing PD.

Methods: We used data from the Singapore Chinese Health Study, a population-based prospective cohort of 63,257 men and women aged 45 to 74 years during enrollment in 1993–1998. Antioxidant intake was derived from a validated semiquantitative food frequency questionnaire. Incident cases were identified through follow-up interviews, hospital records, or PD registries through 31 July 2018. Hazard ratios and corresponding 95% confidence intervals were derived from multivariable Cox proportional hazard regression models with adjustment for other lifestyle and dietary factors.

Results: During an average 19.4 years of follow-up, 544 incident PD cases were identified. No association was found for dietary carotenoids, individually or summed. Hazard ratio comparing highest to lowest quartile for total carotenoids was 0.98 (95% confidence interval: 0.76–1.28; P_{trend} = 0.83). There were also no clear dose-dependent associations of dietary vitamins A, C, and E with risk of developing PD (all $P_{trend} \geq 0.10$). Sensitive analyses with lag time and excluding supplement use did not materially alter results.

Conclusions: Intake of dietary antioxidants, such as carotenoids and vitamins, was not associated with the risk of developing PD in Singaporean Chinese. © 2020 The Authors. *Movement Disorders* published by Wiley Periodicals LLC on behalf of International Parkinson and Movement Disorder Society.

Key Words: antioxidants; diet; oxidative stress; Parkinson's disease; prospective study

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Relevant conflicts of interest/financial disclosures: Nothing to report.

Full financial disclosures and author roles may be found in the online version of this article.

Funding agencies: This study was supported by the National Institutes of Health (R01 CA144034 and UM1 CA182876) and the Singapore Ministry of Health's National Medical Research Council under its Translational and Clinical Research Flagship Programme (NMRC/TCR/013-NNI/2014) and Open Fund Large Collaborative Grant (MOH-OFLCG18May-0002).

Received: 27 February 2020; **Revised:** 24 April 2020; **Accepted:** 26 May 2020

Published online 9 July 2020 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.28173

Parkinson's disease (PD) is a chronic progressive neurodegenerative disorder and one of the fastest-growing reasons for disability among the elderly in the world.[1] However, many of the known risk factors, such as genetic mutations, male sex, and old age, are not modifiable.[2] As such, the hunt is ongoing for potential lifestyle factors that can reduce the risk of developing PD.[3]

In the search for modifiable factors that may modulate PD risk, a logical starting point is the molecular mechanisms underlying PD. At the cellular level, PD results from the dysfunction of dopaminergic neurons, and one of the main theories for the cause of this dysfunction, is oxidative stress.[4] There are several reasons for this. First, neurons have higher energy requirements and thus contain more mitochondria than an average cell. This, in turn, increases their production of reactive oxygen species[4] and renders them highly susceptible to oxidative stress. Dopaminergic neurons are particularly at risk because dopamine is oxidised to dopamine-quinones,[5] which can then bind to potent antioxidants, such as superoxide dismutase 2, to inactivate them,[6] as well as to bind to α-synuclein protofibrils to cause accumulation of Lewy bodies.[7] Furthermore, many of the genetic mutations that have been linked to PD have been shown to disrupt physiological antioxidant mechanisms. For instance, studies have shown that excess α-synuclein (SNCA; PARK1) inhibits complex I of mitochondria,[8] whereas leucine-rich repeat kinase 2 (LRRK2; PARK8) is critical for mitophagy.[9] There is also evidence that daisuke-junko-1 (DJ-1; PARK7) has innate antioxidant properties,[10] and its loss reduces the transcription of antioxidative genes.[11]

Thus, it is logical to hypothesise that reducing oxidative stress may be a potentially viable means of reducing PD risk. Indeed, preclinical studies of antioxidants in PD animal models have been promising, where rats injected with antioxidants were seen to exhibit reduced dopaminergic neuron death after induction of mitochondrial stress.[12] More pertinently, rats fed with dietary antioxidants instead of normal chow also appear to be less susceptible to the death of dopaminergic neurons in the SN after injection of neurotoxin.[13,14]

Many epidemiological studies have investigated nutritional intake based on food frequency questionnaires (FFQs), which are still one of the main dietary assessment tools. However, evidence for a neuroprotective effect of dietary antioxidants on PD risk has, so far, been conflicting. Several studies have suggested that vitamin E,[15-19] vitamin C,[18] or β-carotene[15,16,18] may reduce the risk of PD, whereas there is no association in other studies.[20-24] In comprehensive meta-analyses, vitamin E has been suggested to be protective against PD,[25] but there is no association between carotenoids or vitamin C and PD risk.[25,26] To further confuse matters, the U.S.-based cohorts of the NHS (Nurses' Health Study) and HPFS (Health Professionals Follow-Up Study) have shown conflicting results.[19,21,27]

To the best of our knowledge, no prospective cohort study on dietary antioxidants and PD risk has been conducted in any Asian population. Given that there are significant differences in diet between Western and Asian populations,[28,29] it is reasonable to conclude that there may be differences in sources and quantities of dietary antioxidants consumed and therefore epidemiological differences between these two major types of populations. Furthermore, given that FFQs must be specific to study populations for maximal accuracy,[30,31] there is a need for more cohort studies conducted in heterogeneous populations using FFQs previously validated for accuracy and precision.

Therefore, we prospectively examined the relationship between FFQ-based estimates of dietary antioxidants intake and risk of PD in a 63,257-strong cohort of middle-aged and elderly Chinese men and women in Singapore. These antioxidants included the carotenoids, such as α-carotene, β-carotene, lycopene, β-cryptoxanthin, and lutein, as well as vitamins, such as vitamins A, C, and E. Earlier results from this cohort have previously been published, including associations between FFQ estimates of caffeine, fatty acids, and cholesterol with PD risk,[32,33] and we now present data after a further 13 years of follow-up and with >500 incident PD cases.

Participants and Methods

Study Population

The SCHS (Singapore Chinese Health Study) is a prospective, population-based cohort that was established by the recruitment of 63,257 ethnic Chinese between April 1993 and December 1998. The study recruited only citizens or permanent residents aged 45 to 74 years, residing in government-built housing estates (86% of the population resided in such estates during the enrollment period), and belonging to one of the two major dialect groups of either Hokkien Chinese or Cantonese Chinese.[34] This study was approved by the institutional review boards of the National University of Singapore, the Singapore Health Services, and the National Healthcare Group. All participants gave informed consent.

Assessment of Dietary Antioxidants and Covariates

At recruitment, trained interviewers conducted in-person interviews in the homes of participants using a structured questionnaire to obtain information on demographics, medical history, smoking history, menstrual and reproductive histories (for women), current physical activity, and occupational exposure. This

interview also included a semiquantitative FFQ specifically developed for our study population to determine the participants' dietary habits over the past year. It contained 165 distinct food items common in the Singapore Chinese diet, comprehensively identified from 400 person-days of food intake recorded from 200 persons in a pilot study.[34]

For each food item, participants referred to accompanying photographs to select from eight intake frequency categories (ranging from "never or hardly ever" to "two or more times a day") and three portion sizes (small, medium, or large). Participants were also asked whether they took supplements at least weekly for selected vitamins and minerals, including vitamin A, β-carotene, vitamin C, and vitamin E, and, if so, data on frequency and dosage were also collected. Estimated mean daily nutrient intakes were computed based on the Singapore Food Composition Table, which was a food-nutrient database that listed the levels of 96 nutritional and non-nutritional components per 100 g of the food item in the FFQ. This database was created specifically for this cohort and previously described.[34] Briefly, this database utilized data published by the U.S. Department of Agriculture, supplemented with multiple resources such as published food composition tables from the People's Republic of China, Malaysia, and Taiwan. For several cooked items, we began with the raw values from the Chinese Food Composition Table and developed item-specific formulae to adjust published raw values to the cooked state before inclusion in the Singapore database. We also included analyzed values of several nutrients that could not be found in established databases.[34]

The FFQ was subsequently validated against a series of 24-hour dietary recall interviews and its readministration among a subset of 810 participants.[34] Both methods showed similar distributions, with most mean pairs for energy and nutrients within 10% of each other's values. Correlation coefficients for energy intake and selected nutrients from the questionnaire versus the 24-hour recalls ranged from 0.24 to 0.79, which is comparable with a previous validation study in a multiethnic cohort in the United States.[35]

Ascertainment of PD Cases

Identification of PD cases among cohort participants was previously described.[32,33] In brief, potential cases were identified from three independent sources. (1) Three follow-up interviews were conducted on all surviving cohort participants in 1999–2004 (n = 52,322), 2006–2010 (n = 39,528), and 2014–2016 (n = 17,105). Participants were asked whether they had ever been diagnosed by a physician to have PD and, if yes, age at which the diagnosis was ascertained. (2) Record linkage of the cohort database with the government-established nationwide hospital discharge database was carried out with computer assistance. All diagnoses with the International Classification of Diseases, Ninth Revision (ICD-9) code 332 (PD) from 1990 to 2018 in public and private hospitals were identified. (3) Record linkage of the cohort database with three hospital-based PD registries in Singapore through 31 July 2018 was carried out by database linkage. These PD registries listed patients with a diagnosis of PD and who were followed up primarily as outpatients in PD centers housed in the three largest public hospitals in Singapore.

Of the 941 PD cases ascertained from the three sources, 207 cases were identified from source 1, 428 cases from source 2, and 306 cases from source 3. All available medical records of these identified cases were reviewed by a movement disorder fellow (S.K., Y.W., and others) or specialist (L.C.T.) to verify the date of diagnosis and confirm that the diagnosis was primary PD according to the criteria defined by the Advisory Council of the National Institute of Neurological Disorders and Stroke (NINDS).[36] Among them, 53 were prevalent cases and 1 patient had incomplete medical records to ascertain date of diagnosis, whereas 316 patients did not meet our diagnostic criteria of PD. These were not counted as cases. Of the 571 verified incident PD cases, 312 (55%) satisfied NINDS' definition of probable PD, and 208 (36%) satisfied NINDS' definition of possible PD. Thirty-seven cases (6%) met the basic criteria for PD as defined by the presence of two of the following: rest tremor, bradykinesia, rigidity, or asymmetrical onset, of which one had to be either rest tremor or bradykinesia, together with a sustained substantial response to levodopa or dopamine agonist or an insufficient trial of L-dopa or dopamine agonist, in the absence of other causes of parkinsonism. In addition, for 14 (3%) patients, there was clear documentation of PD by a certified internist, but the accessible medical records did not allow for the above evaluation.

Statistical Analysis

From the cohort, we excluded 1,931 participants who were prevalent cases of cancer at recruitment, because of considerations of potential dietary habit changes after cancer diagnosis, as well as 459 men and 564 women with extreme values of daily caloric consumption (<700 or > 3,700 kcal/d for men, <600 or > 3,000 kcal/d for women). Regular record linkage of the cohort database with the Singapore Registry of Births and Deaths was carried out to ascertain the vital status of participants. The final analysis included 60,249 eligible cohort participants, of which 544 were incident PD cases.

For each participant, person-years were counted from the date of recruitment interview to the date of PD diagnosis, date of death, or 31 July 2018, whichever came first. The χ^2 test (for categorical variables) or Student's *t* test (for continuous variables) was used to examine the difference between PD cases and non-PD cases. FFQ-based estimates of intake for each dietary antioxidant, including supplements, were adjusted for total energy intake using the residual method. This removes variations caused by differences in total quantity of food intake, thus allowing the dietary nutrient composition to be directly evaluated.[37] Quartile levels of each energy-adjusted nutrient were derived from the respective distributions among all participants in the cohort.

Proportional hazards regression methods were used to examine the exposure-PD associations with adjustment for potential confounders. The strength of a given association was measured by the hazard ratio (HR), its corresponding 95% confidence interval (CI), and two-sided *P* value. Potential confounders were either factors associated with the risk of PD in this population or established risk factors in published literature. All regression models were adjusted for age at recruitment (years), year of interview (1993–1995, 1996–1998), sex (whole cohort analysis), dialect group (Cantonese, Hokkien), level of education (no formal education, primary school, secondary school, or higher), daily energy intake (kcal), body mass index (<20, 20–<24, 24–<28, or 28+ kg/m^2), cigarette smoking (never, former, or current), black tea intake (none/monthly, weekly, or daily), caffeine intake (quartiles), cholesterol intake (quartiles), and monounsaturated fat intake (quartiles). Quartile categories were determined by intake across the entire cohort. Black tea, cholesterol, and monounsaturated fat intake were included as covariates given that these were previously reported to be independently associated with PD in this study population.[32,33]

TABLE 1. Baseline characteristics of cohort members without PD and PD cases (mean [SD] or percent), The Singapore Chinese Health Study

	PD Cases (n = 544)	Non-PD Subjects (n = 59,705)	*P* Value
Age (years) at recruitment	59.7 (7.6)	56.4 (8.0)	<0.0001
Body mass index (kg/m^2)	23.0 (2.9)	23.1 (3.3)	0.51
Sex (%)			
Males	275 (50.5)	26,534 (44.4)	0.004
Females	269 (49.4)	33,171 (55.6)	
Dialect (%)			
Cantonese	232 (42.6)	27,667 (46.3)	0.09
Hokkien	312 (57.4)	32,038 (53.7)	
Level of education (%)			
No formal education	136 (25.0)	16,124 (27.0)	0.17
Primary school (1–6 years)	264 (48.5)	26,555 (44.5)	
Secondary school and above	144 (26.5)	17,026 (28.5)	
Smoking status (%)			
Never smoker	406 (74.6)	41,467 (69.4)	<0.0001
Former smoker	73 (13.4)	6,497 (10.9)	
Current smoker	65 (12.0)	11,741 (19.7)	
Black tea intake (%)			
None/monthly	395 (72.6)	42,894 (71.8)	0.67
Weekly	95 (17.5)	10,163 (17.0)	
Daily	54 (9.9)	6,648 (11.1)	
Total energy (kcal/d)	1,535.0 (529.3)	1,548.2 (519.9)	0.55
Dietary intake (energy-adjusted)			
Caffeine (mg/d)	126.0 (105.1)	148.3 (108.7)	<0.0001
Cholesterol (mg/d)	156.6 (91.4)	172.3 (100.6)	<0.0001
Monounsaturated fatty acids (g/d)	14.3 (6.7)	14.8 (6.7)	0.11
α-carotene (μg/d)	247.8 (252.9)	254.7 (277.9)	0.52
β-carotene (μg/d)	2,087.3 (1,287.6)	2,154.5 (1,433.6)	0.23
Lycopene (μg/d)	1,001.5 (1,245.9)	1,104.6 (1,484.2)	0.06
β-cryptoxanthin (μg/d)	272.7 (373.0)	252.2 (329.4)	0.20
Lutein (μg/d)	1,815.2 (1,013.7)	1,865.9 (1,100.9)	0.25
Total carotenoids (μg/d)	5,424.5 (3,221.2)	5,631.9 (3,550.2)	0.14
Vitamin A (IU/d)	4,994.7 (2,984.3)	5,085.0 (3,251.1)	0.48
Vitamin C (mg/d)	103.0 (123.6)	102.5 (133.7)	0.92
Vitamin E (mg/d)	12.7 (39.3)	12.5 (47.2)	0.94

P value by chi-square or Student's *t* test.

Tests for trend were performed by using the median values of intake in the quartile categories as continuous variables in the Cox regression models. Heterogeneity of the diet-PD risk associations between men and women was tested by including the product term between sex and median values of the nutrient intake as an interaction term in the Cox model.

All statistical analysis was conducted using SAS software (version 9.2; SAS Institute Inc., Cary, NC). All reported *P* values are two-sided; $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics of participants are presented in Table 1. A total of 544 incident PD cases were observed after a mean follow-up of 19.4 (standard deviation [SD]: 6.2) years. Their mean age at diagnosis was 71.2 (SD, 7.3) years, and the mean time interval between cohort enrollment and PD diagnosis was 10.9 (SD, 6.0) years. The incidence rate of PD, adjusted to the age structure of the whole cohort (aged 45–74 years), was 56.0 per 100,000 person-years in

TABLE 2. Dietary carotenoids in relation to risk of PD, The Singapore Chinese Health Study 1993–2016

	Total		Men		Women	
Energy-adjusted intake by quartile	Cases	HR (95% CI)	Cases	HR (95% CI)	Cases	HR (95% CI)
α-carotene (μg/d)						
1st (<100)	116	1.00	81	1.00	35	1.00
2nd (100–179)	148	1.23 (0.95–1.58)	68	1.12 (0.80–1.56)	80	1.36 (0.90–2.04)
3rd (179–311)	155	1.28 (1.00–1.65)	65	1.11 (0.79–1.56)	90	1.49 (1.00–2.22)
4th (>311)	125	1.06 (0.81–1.37)	61	1.07 (0.76–1.50)	64	1.09 (0.72–1.66)
P for trend (median)		0.77		0.82		0.49
P for interaction		0.29				
β-carotene (μg/d)						
1st (<1,358)	127	1.00	87	1.00	40	1.00
2nd (1,358–1,902)	154	1.18 (0.92–1.50)	71	1.07 (0.77–1.47)	83	1.30 (0.89–1.92)
3rd (1,902–2,674)	142	1.14 (0.89–1.46)	59	1.00 (0.71–1.40)	83	1.30 (0.88–1.91)
4th (>2,674)	121	1.02 (0.78–1.32)	58	1.05 (0.74–1.49)	63	1.02 (0.67–1.54)
P for trend (median)		0.82		0.85		0.54
P for interaction		0.24				
Lycopene (μg/d)						
1st (<420)	139	1.00	73	1.00	66	1.00
2nd (420–794)	140	1.01 (0.78–1.29)	68	1.26 (0.89–1.79)	72	0.77 (0.53–1.10)
3rd (794–1,343)	145	1.15 (0.89–1.48)	69	1.40 (0.99–1.98)	76	0.89 (0.62–1.29)
4th (>1,343)	120	1.02 (0.79–1.31)	65	1.18 (0.83–1.66)	55	0.82 (0.56–1.20)
P for trend (median)		0.84		0.50		0.58
P for interaction		0.44				
β-cryptoxanthin (μg/d)						
1st (<84)	126	1.00	75	1.00	51	1.00
2nd (84–167)	119	0.89 (0.68–1.15)	56	0.90 (0.63–1.28)	63	0.88 (0.60–1.30)
3rd (167–304)	154	1.19 (0.93–1.52)	68	1.07 (0.76–1.51)	86	1.29 (0.90–1.86)
4th (>304)	145	1.06 (0.82–1.35)	76	1.02 (0.73–1.42)	69	1.08 (0.74–1.57)
P for trend (median)		0.41		0.73		0.48
P for interaction		0.89				
Lutein (μg/d)						
1st (<1,231)	136	1.00	86	1.00	50	1.00
2nd (1,231–1,686)	147	1.09 (0.86–1.39)	64	1.00 (0.72–1.39)	83	1.13 (0.79–1.61)
3rd (1,686–2,322)	124	0.96 (0.75–1.24)	62	1.08 (0.77–1.50)	62	0.83 (0.57–1.22)
4th (>2,322)	137	1.12 (0.87–1.44)	63	1.16 (0.82–1.63)	74	1.05 (0.72–1.54)
P for trend (median)		0.52		0.36		0.94
P for interaction		0.17				
Total carotenoids (μg/d)						
1st (<3,722)	133	1.00	86	1.00	47	1.00
2nd (3,722–5,091)	143	1.08 (0.84–1.37)	65	0.99 (0.71–1.38)	78	1.15 (0.79–1.66)
3rd (5,091–6,929)	152	1.21 (0.94–1.54)	63	1.06 (0.76–1.49)	89	1.33 (0.92–1.92)
4th (>6,929)	116	0.98 (0.76–1.28)	61	1.08 (0.76–1.51)	55	0.90 (0.60–1.35)
P for trend (median)		0.91		0.63		0.45
P for interaction		0.17				

Adjusted for age at recruitment, year of interview, sex, dialect group, level of education, energy, body mass index, cigarette smoking, black tea intake, caffeine intake, cholesterol intake, and monounsaturated fat intake.

TABLE 3. Dietary vitamins in relation to risk of PD, The Singapore Chinese Health Study 1993–2016

Energy-adjusted intake (quartile median)	Total Cases	HR (95% CI)	Men Cases	HR (95% CI)	Women Cases	HR (95% CI)
Vitamin A (IU/d)						
1st (<3,322)	127	1.00	86	1.00	41	1.00
2nd (3,322–4,480)	144	1.12 (0.87–1.43)	71	1.12 (0.81–1.55)	73	1.09 (0.74–1.61)
3rd (4,480–6,180)	150	1.21 (0.95–1.56)	54	0.96 (0.67–1.36)	96	1.42 (0.97–2.07)
4th (>6,180)	123	1.01 (0.78–1.32)	64	1.15 (0.82–1.62)	59	0.89 (0.59–1.35)
P for trend (median)		0.92		0.52		0.36
P for interaction		0.09				
Vitamin C (mg/d)						
1st (<52.1)	117	1.00	64	1.00	53	1.00
2nd (52.1–77.1)	147	1.25 (0.97–1.61)	73	1.50 (1.06–2.12)	74	1.00 (0.70–1.45)
3rd (77.1–115.6)	130	1.14 (0.88–1.48)	66	1.30 (0.91–1.86)	64	0.94 (0.65–1.38)
4th (>115.6)	150	1.30 (1.01–1.67)	72	1.33 (0.94–1.89)	78	1.20 (0.83–1.73)
P for trend (median)		0.10		0.27		0.26
P for interaction		0.85				
Vitamin E (mg/d)						
1st (<4.8)	121	1.00	90	1.00	31	1.00
2nd (4.8–6.2)	167	1.41 (1.08–1.83)	91	1.40 (1.00–1.96)	76	1.31 (0.83–2.06)
3rd (6.2–7.4)	127	1.14 (0.83–1.54)	45	0.97 (0.64–1.49)	82	1.17 (0.72–1.90)
4th (>7.4)	129	1.23 (0.90–1.70)	49	1.26 (0.83–1.91)	80	1.18 (0.71–1.97)
P for trend (median)		0.38		0.41		0.83
P for interaction		0.26				

Adjusted for age at recruitment, year of interview, sex, dialect group, level of education, energy, body mass index, cigarette smoking, black tea intake, caffeine intake, cholesterol intake, and monounsaturated fat intake.

men and 39.8 per 100,000 person-years in women. Compared with the rest of the cohort, incident PD cases were significantly older at recruitment, more likely to be men, less likely to be smokers, consumed less caffeine-containing beverages such as coffee and tea, and consumed less dietary cholesterol.

For dietary carotenoids (Table 2), we found no significant association between total carotenoid intake and PD risk (P_{trend} = 0.91). This was true for both men (P_{trend} = 0.63) and women (P_{trend} = 0.45). There was no association between individual carotenoid intake (ie, α-carotene, β-carotene, lycopene, β-cryptoxanthin, and lutein and PD risk). There was also no association found between FFQ-based estimates of intake of vitamin A (P_{trend} = 0.92), vitamin C (P_{trend} = 0.10), or vitamin E (P_{trend} = 0.38) and PD risk (Table 3). Results were comparable between men and women, and tests for interaction for all dietary antioxidants were not significant.

Only 6.3% of our study population took a dietary antioxidant supplement at least weekly. Specifically, only 2.1% took vitamin A, 0.1% took β-carotene, 3.9% took vitamin C, and 3.0% took vitamin E supplements. In sensitivity analysis for diet-only intake, there were no material alterations to our results. No significant association was noted in total carotenoids (HR comparing extreme quartiles: 0.99; 95% CI: 0.76–1.28; P_{trend} = 0.94), vitamin A (HR comparing extreme quartiles: 0.98; 95% CI: 0.75–1.29; P_{trend} = 0.81), vitamin C (HR comparing extreme quartiles: 1.26; 95% CI: 0.97–1.64; P_{trend} = 0.18), or vitamin E (HR comparing extreme quartiles: 1.16; 95% CI: 0.82–1.64; P_{trend} = 0.59) and risk of PD. Results remained comparable in a lag analysis that excluded those with <4 years of follow-up (data not shown).

Discussion

In this population-based cohort of middle-aged and elderly Chinese in Singapore, we studied carotenoids that included α-carotene, β-carotene, lycopene, β-cryptoxanthin, and lutein and vitamins that included vitamin A, vitamin C, and vitamin E and did not find any association between FFQ-based estimates of these dietary antioxidants and PD risk.

Oxidative stress has been a major area for preclinical research in PD. Many animal studies have tested extracts from various common edible plants with antioxidant activity, such as ginseng,[38] turmeric,[39] and cordyceps sinensis,[40] in reducing onset or symptoms in animal models of PD. Others have focused on antioxidative vitamins, such as vitamin C[41] and vitamin E,[12,42] and found them to reduce the development of PD in these experimental models. However, the majority of these studies have limitations in their design for direct human applications: Animals were either directly injected with the antioxidants or were fed concentrated extracts involving quantities far exceeding normal human consumption levels. Furthermore, these antioxidants were either given near the time point of neuronal insult or in

animal gene-knockout models, neither of which could encapsulate the usual progression of PD in humans.

Indeed, although many epidemiological studies have examined the associations between intake of dietary antioxidants and PD risk, results have been highly inconsistent. In cross-sectional and case-control studies, no statistically significant association was found between intake of vitamin A,[20,22,43,44] vitamin C,[15,16,20,22,43-45] or α-carotene[16,45] and PD risk. For vitamin E, although the majority of studies did not find any association with PD risk,[20,22,43-45] three studies showed an inverse relationship.[15-17] Schirinzi and colleagues went on to test dietary antioxidants in a phosphatase and tensin homolog-induced kinase 1 knockout ($PINK1^{-/-}$) mouse model, and found that vitamin E was able to restore synaptic plasticity both ex vivo and in vivo, but vitamin A, vitamin C, β-carotene, lycopene, and lutein were all unable to do so.[17] For β-carotene, two studies reported an association with reduced PD risk,[15,16] but, in most studies, results were null.[44-46] However, a positive association between lycopene and PD risk had been reported in one study,[46] although another study found that lycopene reduced PD risk.[45] Another case-control study reported a positive association between β-cryptoxanthin and PD risk,[45] but two other case-control studies found no association.[16,46] There were also two studies that found lutein to increase the risk of PD,[44,45] but another study did not find any association.[46] Given the intrinsic propensity for reverse causality, recall bias, and selection bias in retrospective and cross-sectional studies, it is difficult to interpret these results.

In prospective cohort studies that examined the relationship between antioxidants and risk of PD, most of them have found no statistically significant association.[21,23,24] Despite 30 years of follow-up, investigators did not find any association between vitamin E intake and PD risk in the Honolulu Heart Study Cohort.[23] In another nested case-control study from the U.S.-based Leisure World Cohort, there was also no association found between intake of vitamin A, vitamin C, vitamin E or β-carotene and risk of PD.[24] More recently, in the NHS and HPFS cohorts, investigators found no association between intake of dietary carotenoids (α-carotene, β-carotene, lycopene, β-cryptoxanthin, lutein, or total), vitamin C, or vitamin E and PD risk.[21] All of these are consistent with our findings from the SCHS.

However, there were two cohort studies that found inverse relations between dietary antioxidant intake and PD risk.[18,19] In 2002, Zhang and colleagues had previously reported an association between moderate vitamin E intake and PD risk in the NHS and HPFS cohorts, but this effect was not in a dose-dependent manner.[19] Furthermore, this association was not present in the follow-up study in these cohorts in 2016, and Hughes and colleagues have suggested that their previous finding could have been attributable to chance.[21] In another study conducted on two Swedish cohorts, Yang and colleagues reported an inverse dose-dependent association of borderline significance for vitamin C with PD risk in women only, statistical significance for vitamin E in women, and statistical significance for β-carotene in both men and women.[18] This discrepancy with our results may have a few explanations. First, Yang and colleagues used ICD codes in registry data, with a positive predictive value of 70.8% and a sensitivity of 72.7%. On the other hand, all incident PD cases in the SCHS were confirmed by manual review of notes, with a positive predictive value of up to 95.4%.[47] As such, the Swedish study may have included a significant number of patients who have other forms of parkinsonism instead of PD. In addition, it is also possible that the difference in our findings from theirs were a result of the dietary intake levels in the Swedish cohort being higher compared to those in our cohort. Nevertheless, it is noteworthy that despite even higher intake levels in the NHS and HPFS cohorts than those in the Swedish cohorts, these two U.S. cohorts reported null associations.[21]

There are potential biological explanations for our null results. Despite being a potent antioxidant, dietary vitamin C may not affect the risk of PD because it lowers serum uric acid levels,[48] and urate has been previously shown to be another potent antioxidant that reduces the risk of PD.[49-52] As such, it is possible that the hypouricaemic effects of vitamin C may have attenuated its own antioxidant effects. Another potent antioxidant, vitamin E, has been shown to have no effect on PD progression in humans. In the DATATOP (Deprenyl And Tocopherol Antioxidative Therapy Of Parkinsonism) randomized controlled clinical trial,[53] although alpha-tocopherol was able to cross the blood–brain barrier,[54] it had no effect on the activity of nigrostriatal dopaminergic neurons[55] and hence no effect on the progression of PD.[56] Furthermore, in most preclinical studies that showed an association between antioxidants and PD risk, animals were either directly injected intraperitoneally with antioxidants or they were fed highly concentrated extracts. It is therefore possible that the dietary antioxidants we examined were not consumed in sufficient quantities in the Singaporean diet to have measurable effects in reducing PD risk in the population.

The main strengths of this study include the prospective design, the long follow-up period, the large sample size, the use of validated dietary assessments developed specifically for this cohort, dietary exposure as the predominant source of antioxidants attributed to low prevalence of supplement users in this cohort, and case ascertainment by trained medical specialists. Nevertheless, we do acknowledge that there are several limitations to our study. Some misclassification in the

assessment of nutrient intake and residual confounding is inevitable, whether attributed to the FFQ, dietary changes over time after baseline interview, or self-reporting of lifestyle-related data and baseline comorbidity status. All of these could result in an underestimation of the true effect size of dietary antioxidants on the risk of PD. Furthermore, our study utilizes FFQ-based estimates of dietary intake, which could be inaccurate because of self-reporting and undercapturing of food items.[31,57] However, we have minimized the impact of these limitations by developing a questionnaire specific to the SCHS and validating it to ensure its accuracy and precision.[34] Moreover, even though the FFQ is known to underestimate true intake levels in a systematic way in a cohort, FFQ-based dietary assessment is still capable of ranking persons' dietary exposures and studying associations between their relative intake and disease risk.[58] As such, conclusions drawn from FFQ-based studies are directly translatable into public health education in the form of dietary advice to the general public, given that such advice is usually in terms of encouraging or discouraging intake of specific dietary nutrients rather than prescribing absolute quantity.

In conclusion, in this large, population-based, prospective cohort in an Asian population, we did not find any significant association between FFQ-based estimates of dietary intake of vitamin A, vitamin C, vitamin E, or common carotenoids with PD risk. These results are in line with most other prospective studies conducted in other populations. As such, even though there is a strong biological plausibility underlying promising findings from experimental studies, more research in human studies is necessary to determine whether increasing the consumption of dietary antioxidants or the use of such supplements at midlife is a viable modification to reduce the risk of PD in aging populations. ●

Acknowledgments: We are grateful to Ms. Siew-Hong Low of the National University of Singapore for supervising the fieldwork of the Singapore Chinese Health Study. We acknowledge the founding Principal Investigator of the Singapore Chinese Health Study, Mimi C. Yu. We also thank the Ministry of Health in Singapore for assistance with the identification of PD cases by record linkage analysis with various databases. Finally, we acknowledge the following doctors for their assistance to access medical records: Drs. June Tan (National University Health System), Kay-Yaw Tay (Khoo Teck Puat Hospital), David Ng (National Healthcare Group Polyclinics), and Ngiap-Chuan Tan (Singhealth Polyclinics).

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