Vegetarian diet and incidence of total, ischemic, and hemorrhagic stroke in 2 cohorts in Taiwan

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Abstract

Objective
To determine how a vegetarian diet affects stroke incidence in 2 prospective cohorts and to explore whether the association is modified by dietary vitamin B_{12} intake.

Methods
Participants without stroke in the Tzu Chi Health Study (cohort 1, n = 5,050, recruited in 2007–2009) and the Tzu Chi Vegetarian Study (cohort 2, n = 8,302, recruited in 2005) were followed until the end of 2014. Diet was assessed through food frequency questionnaires in both cohorts at baseline. Stroke events and baseline comorbidities were identified through the National Health Insurance Research Database. A subgroup of 1,528 participants in cohort 1 were assessed for serum homocysteine, vitamin B_{12}, and folate. Associations between vegetarian diet and stroke incidences were estimated by Cox regression with age as time scale, adjusted for sex, education, smoking, alcohol, physical activities, body mass index (only in cohort 1), hypertension, diabetes, dyslipidemia, and ischemic heart diseases.

Results
Vegetarians had lower serum vitamin B_{12} and higher folate and homocysteine than non-vegetarians. In cohort 1, 54 events occurred in 30,797 person-years follow-up. Vegetarians (vs nonvegetarians) experienced lower risk of ischemic stroke (hazard ratio [HR], 0.26; 95% confidence interval [CI], 0.08–0.88). In cohort 2, 121 events occurred in 76,797 person-years follow-up. Vegetarians (vs nonvegetarians) experienced lower risk of overall stroke (HR, 0.52; 95% CI, 0.33–0.82), ischemic stroke (HR, 0.41; 95% CI, 0.19–0.88), and hemorrhagic stroke (HR, 034; 95% CI, 0.12–1.00). Our explorative analysis showed that vitamin B_{12} intake may modify the association between vegetarian diet and overall stroke (*p* interaction = 0.046).

Conclusion
Taiwanese vegetarian diet is associated with a lower risk of ischemic and hemorrhagic strokes.

Glossary

BMI = body mass index; **BP** = blood pressure; **CI** = confidence interval; **eGFR** = estimated glomerular filtration rate; **FFQ** = food frequency questionnaire; **HR** = hazard ratio; **HWDC** = Health and Welfare Data Science Center; **ICD-9-CM** = International Classification of Diseases, 9th Revision, Clinical Modification; **LDL** = low-density lipoprotein; **NHIP** = National Health Insurance Program; **NHIRD** = National Health Insurance Research Database; **RDA** = recommended dietary allowance; **SBP** = systolic blood pressure; **TMAO** = trimethylamine N-oxide.

Stroke is the second most common cause of death globally, accounting for about 1 out of every 19 deaths in the United States,[1] and is the third leading cause of death in Taiwan.[2] In Taiwan, ischemic subtype accounts for 74% of all stroke, followed by intracranial hemorrhage (16.1%), TIA (6.7%), subarachnoid hemorrhage (2.8%), and cerebral venous thrombosis (0.2%).[2] Stroke is also the most common cause of complex disability and an independent risk for dementia.[3] The direct and indirect cost of stroke care is a considerable burden to both the society and patients' families.

Although some risk factors of stroke are nonmodifiable, such as older age and male sex, current known modifiable risk factors—hypertension, current smoking, high waist-to-hip ratio, diet, regular physical activity, diabetes mellitus, alcohol consumption, psychological factors, cardiac causes, ApoB/Apo1 ratio—together account for more than 90% of population attributable risk for stroke.[2,4] In addition to these risk factors, metabolic syndrome, dyslipidemia, and high low-density lipoprotein cholesterol have been identified as risk factors for intracranial atherosclerosis, an important mechanistic step in the pathogenesis of ischemic stroke.[5]

Vegetarian diets have been documented to lower blood pressure (BP), glucose, and cholesterol,[6–8] and reduce cardiovascular disease incidence and mortality.[9,10] Other dietary patterns that emphasized plant foods have been shown to be protectively associated with stroke incidence.[11–14] However, vegetarians tend to have low intake of vitamin B_{12}, and this could raise homocysteine, leading to an increase in risk of stroke.[15] In addition, previous studies suggested that some animal protein may be beneficial for the prevention of hemorrhagic stroke[16] or improved cerebral infarction mortality.[17] The combined effect of the supposed harm of high homocysteine and the supposed benefits of low BP, glucose, and cholesterol (unique aspect of vegetarian diets) on stroke disease incidence is unclear. A previous pool analysis showed that the association between vegetarian diet and cerebrovascular mortality trends toward protection in men (hazard ratio [HR], 0.77; 95% confidence interval [CI], 0.57, 1.02) but not in women (HR, 0.98; 95% CI, 0.80, 1.20), though overall association is heterogeneous in different studies.[18] Moreover, many studies looking into diet and stroke incidence tend to lack information on BP, glucose, and cholesterol, making it difficult to examine whether the effect of diet is mediated through these relevant risk factors.

We investigated the incidence of overall, ischemic, and hemorrhagic stroke in vegetarians and nonvegetarians in 2 prospective cohorts in Taiwan. In addition, we explored whether the association between vegetarian diet and stroke risk is modified by vitamin B_{12} intake. We hypothesized that a vegetarian diet protects against stroke through reduction of metabolic risk factors and that this effect would be particularly strong among vegetarians with adequate intake of vitamin B_{12}.

Methods

Standard protocol approvals, registrations, and patient consents

The 2 cohorts were recruited in different periods and both were approved by the institutional review board at the Buddhist Dalin Tzu Chi General Hospital. All participants gave written informed consent.

The study was retrospectively registered on March 20, 2018. The protocol is available at clinicaltrials.gov (unique identifier: NCT03470584).

Study design and population

The 2 cohort studies, Tzu Chi Health Study (cohort 1) and Tzu Chi Vegetarian Study (cohort 2), were prospectively followed up to identify stroke events through linkage to the National Health Insurance Research Database (NHIRD).

Most of the participants were Tzu Chi volunteers, Buddhists who commit themselves to a variety of community services, ranging from charity to environmental protection. All volunteers went through at least 2 years of training to assure they know the core value of Tzu Chi and quit smoking and drinking alcohol before becoming certified as Tzu Chi volunteers. Vegetarianism is encouraged in this population as a means to promote planetary health and animal welfare. About 30% of the volunteers were full-time vegetarians in both cohorts.

Tzu Chi Health Study (cohort 1)

Cohort 1 recruited 6,002 participants (77% certified Tzu Chi volunteers and 23% their families or individuals who came to the Buddhist Dalin Tzu Chi Hospital for routine health examination) from 2007 to 2009.

All participants received a comprehensive health examination at baseline and were interviewed on a questionnaire for demographics, medical history, lifestyle habits (smoking, alcohol,

leisure time physical activities), and diet (a questionnaire on vegetarian dietary practice [type and duration] and a quantitative food frequency questionnaire [FFQ]). This FFQ (consisting of 64 food/food groups with an additional section on use of supplements) had been validated among Tzu Chi volunteers and showed good reliability and validity; the correlation between FFQ-assessed vitamin B_{12} and serum vitamin B_{12} level is 0.41.[19] Baseline fasting lipid profile, glucose, and creatinine were assessed through the INTEGRA 800 system (Roche Diagnostics, Indianapolis, IN; imprecision: <2.5% coefficient of variation). Estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration. BPs were measured using the VP 1000 System (Colin Co., Ltd., Komaki, Japan). A subgroup of 1,528 cohort members, selected at a ratio of 2:1 for nonvegetarians:vegetarians, contributed additional blood samples for analysis of homocysteine, vitamin B_{12}, and folate. Serum folate and vitamin B_{12} were assessed by a commercial RIA kit (Siemens, Malvern, PA). Serum homocysteine was assessed using ADIVA Centuaur Immunoassay System (Siemens Healthcare Diagnostics Inc., Tarrytown, NY).

Tzu Chi Vegetarian Study (cohort 2)

Cohort 2 consists of 12,062 participants (average age 50 years) recruited from Tzu Chi Foundation branches throughout Taiwan in 2005. Community volunteer leaders in Tzu Chi sites throughout Taiwan assisted in distributing the questionnaire to volunteer members in their communities. The study questionnaire used in cohort 2 was similar to that of cohort 1, except the FFQ had only 57 items, with frequency but no portion size. However, only cohort 1 had anthropometric and biochemical measurements. Cohort 2 lacked such data.

Exposure assessment

Vegetarian status was defined by avoidance of meat and fish in face to face confirmation by trained interviewers and in the FFQ in cohort 1. As for cohort 2, the diet section includes a question inquiring whether the participant was a vegetarian or a nonvegetarian. Participants who identified themselves as vegetarians in this question and reported consuming no meat and fish in the FFQ were classified as vegetarians; those who self-identified as nonvegetarians or reported any consumption of meat or fish within the previous year (in the FFQ) were classified as nonvegetarians.

Disease ascertainment

The cohort data (including demographics and baseline diet and lifestyle) were linked to the NHIRD by a unique identification number under official legal permission. Incident stroke events and other baseline comorbidities were ascertained through NHIRD by the ICD-9-CM.

The National Health Insurance Program (NHIP) is a universal health care program implemented on March 1, 1995, in Taiwan and covers nearly 100% of Taiwan's population (nhird.nhri.org.tw/en/index.html). The NHIRD contains all medical claim records reimbursed by the NHIP and demographic information, such as dates of birth, sex, and clinical diagnoses, which are based on ambulatory clinical visit and inpatient care claims. The data are located in the Health and Welfare Data Science Center (HWDC) and belong to the Ministry of Health in Taiwan.

Incident stroke cases and stroke subtypes were prospectively identified during the 10-year follow-up (up to end of 2014) by the primary diagnosis in the hospitalization records.[20] ICD-9-CM codes were used to identify disease conditions: total stokes (430–438), hemorrhagic stroke (430–432), and ischemic stroke (433–434).

Other underlying comorbidities selected based on prior studies and suspected relationships were also considered: diabetes (250), hypertension (401–405), ischemic heart disease (410–414), and dyslipidemia (272). As the first diagnosis code may be given for further advanced examinations for ruling out purposes, presence of diseases was ascertained by having at least 2 diagnosis records of certain disease in order to prevent false-positives.

Exclusion criteria

We excluded participants who had no NHIRD data, age <20 years, those with missing values for covariates, and those with any stroke records in inpatient or outpatient database at baseline (the inclusion and exclusion scheme of the 2 cohorts is detailed in the figure).

Statistical analysis

Baseline descriptive statistics were compared between vegetarian and nonvegetarian groups using independent t test and χ^2 test. Intakes of foods and nutrients in vegetarians and nonvegetarians were compared using Wilcoxon rank-sum test (nonnormal distribution) and χ^2 test. Wilcoxon rank-sum test and χ^2 tests were used to compare serum homocysteine, vitamin B_{12}, and folate between 406 vegetarians and 877 nonvegetarians.

Cox proportional hazards regression was used to examine the association between diet and stroke risk. Age rather than time on study was used as the time scale, as this is recommended by several statistical methodology articles as more appropriate in dealing with aging-related outcomes.[21,22] For both cohorts, our analyses include crude model, sex-adjusted model, main model (adjusted for sex, education, body mass index [BMI] [only cohort 1], and lifestyle variables [smoking, alcohol drinking habits, betel nut use, and leisure time physical activities]), and final model (adjusted for factors in main model and relevant comorbidities, including hypertension, dyslipidemia, diabetes mellitus, and ischemic heart disease). We included a sensitivity analysis for cohort 1, in which baseline measurements of systolic BP (SBP), fasting glucose, total cholesterol, and triglycerides were included instead of hypertension, diabetes, and dyslipidemia.

We further examined the relationship between diet and subtypes of stroke—ischemic and hemorrhagic—by competing risk analysis with cause-specific hazard function.[23]

Figure Inclusion and exclusion criteria
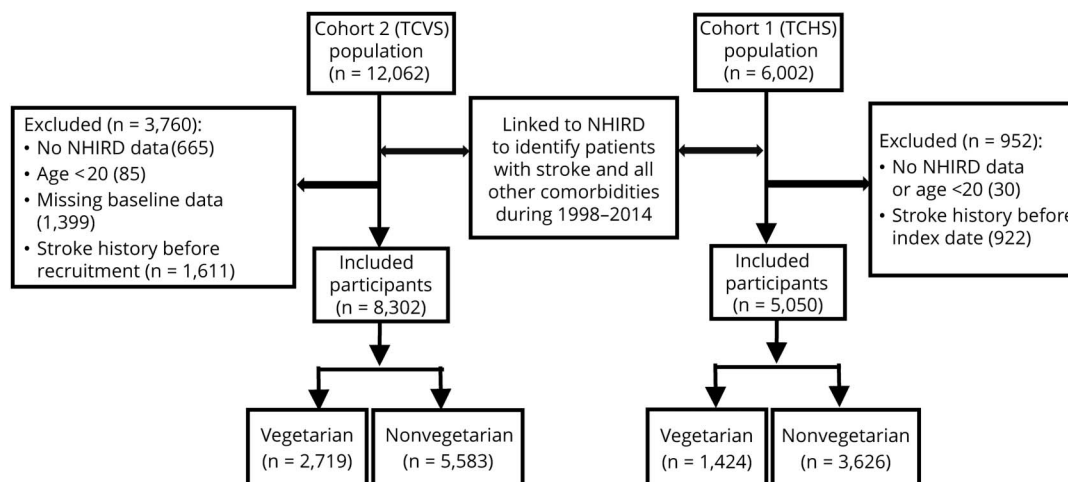
NHIRD = National Health Insurance Research Database; TCHS = Tzu Chi Health Study; TCVS = Tzu Chi Vegetarian Study.

Finally, we conducted a subgroup analysis by vitamin B_{12} intake using the cut point of 2.4 μg, the recommended dietary allowance (RDA) for vitamin B_{12} in Taiwan. Whether the effect of vegetarian diet on stroke risk is modified by vitamin B_{12} was assessed by p value of product between vegetarian diet and vitamin B_{12} in the Cox regression model.

All analyses were performed using SAS, version 9.4 (SAS Institute Inc., Cary, NC) within HWDC.

Data availability
All the dataset is located in the HWDC. As per local law and regulation, NHIRD data can only be accessed and analyzed within the facility of the HWDC. To access these data, one needs to submit an application to the HWDC of the Ministry of Health in Taiwan. All supplementary data are available at doi.org/10.5061/dryad.x95x69pcz.

Results

Baseline characteristics of the 2 cohorts
The baseline characteristics for the 2 cohorts are presented in table 1. Vegetarians had a higher proportion of women, never-smokers, and never-alcohol drinkers, and a lower proportion with elevated SBP (cohort 1) or hypertension (cohort 2), dyslipidemia, regular leisure time physical activities, and low education levels. The mean age was slightly higher in vegetarians, while BMI, cholesterol, and glucose were lower in vegetarians. Only 1.1% of vegetarians and 1.5% of nonvegetarians had abnormal renal function (eGFR <60 mL/min/1.73 m^2).

Food and nutrient intakes estimated by FFQ
Table 2 shows the intake of foods and nutrients (median and interquartile range) in nonvegetarians and vegetarians in cohort 1. Vegetarians consumed more soy, vegetables, and nuts, and less dairy compared with nonvegetarians, while egg

consumption was similar in both groups. For nutrients, vegetarians consumed more dietary fibers and plant protein, but less animal protein, fat, and vitamin B_{12}. The proportion with inadequate intakes of vitamin B_{12} was higher in vegetarians (64%) than in nonvegetarians (33%). The findings were similar when foods and nutrients were standardized to 2,000 kcal, as shown in table e-1 (doi.org/10.5061/dryad.x95x69pcz).

Serum homocysteine, vitamin B_{12}, and folate concentration
In selected participants with serum homocysteine, vitamin B_{12}, and folate data available (406 vegetarians and 877 nonvegetarians) within cohort 1, vegetarians had lower serum vitamin B_{12} and higher serum folate and homocysteine, as indicated by median (table 3). Additional comparison of least square mean of these biomarkers (between vegetarians and nonvegetarians), with adjustment of sex, smoking, and alcohol drinking by general linear model, showed no substantial change (data not shown). Vegetarians were more likely to have elevated homocysteine (25% of vegetarians vs 8% of nonvegetarians had homocysteine ≥14 μmol/L) and vitamin B_{12} deficiency (26% of vegetarians vs 1% of nonvegetarians had serum vitamin B_{12} <200 pg/mL), as shown in table 3. Folate deficiency was rare, and the numbers were too small to be released as per regulation of the HWDC.

Association between vegetarian diet and stroke incidence in cohort 1
There were 54 incident stroke events in the 30,797 person-years of follow-up. Overall stroke incidence in vegetarians was lower than in nonvegetarians (1.16 [0.62–2.15] vs 1.99 [1.48–2.67] per 1,000 person-years). As shown in table 4, vegetarians (vs nonvegetarians) had only about half of the risk of overall stroke throughout various models. SBP and fasting glucose were associated with risk (in sensitivity analysis; table e-2, doi.org/10.5061/dryad.x95x69pcz).

Table 1 Baseline characteristics of vegetarians and nonvegetarians in cohort 1 (Tzu Chi Health Study [TCHS]) and cohort 2 (Tzu Chi Vegetarian Study [TCVS])

Characteristic	Cohort 1 (TCHS)			Cohort 2 (TCVS)		
	Nonvegetarians (n = 3,626)	Vegetarians (n = 1,424)	*p* Value	Nonvegetarians (n = 5,583)	Vegetarians (n = 2,719)	*p* Value
Age, y, mean (SD)	51.7 (10.5)	53.8 (9.03)	<0.01	49.2 (9.6)	50.1 (9.3)	<0.01
Male, %	47.2	25.1	0.05	40.5	27.9	<0.01
Education level, %			<0.01			<0.01
Elementary school or less	20.5	28.4		18.5	22.3	
Secondary school	48.6	49.9		54.0	51.2	
College or higher	30.9	21.7		27.6	26.5	
Ever smoking, %	21.2	8.5	<0.01	16.7	10.2	<0.01
Ever alcohol drinking, %	18.0	8.1	<0.01	15.2	11.0	<0.01
Ever betel nut, %	7.7	3.0	<0.01	6.3	4.8	<0.01
Leisure time physical activities, %	68.7	64.7	<0.01	84.7	80.8	<0.01
Hypertension, %	14.5	14.2	0.79	11.5	9.4	<0.01
Diabetes mellitus, %	5.8	4.6	0.09	13.6	11.8	0.05
Dyslipidemia, %	8.5	5.7	<0.01	6.4	3.8	<0.01
Ischemic heart disease, %	3.9	3.7	0.81	2.9	2.5	0.29
SBP, mean (SD)	126 (16.4)	125 (17.0)	0.02			
Elevated blood pressure, %	29.8	23.4	<0.01			
Fasting glucose, mean (SD)	94.3 (20.0)	92.3 (15.9)	<0.01			
Impaired fasting glucose, %	19.3	14.0	<0.01			
Total cholesterol, mean (SD)	196 (36.4)	180 (33.9)	<0.01			
HDL, mean (SD)	54.4 (14.8)	52.4 (13.7)	<0.01			
LDL, mean (SD)	129 (33.3)	115 (29.4)	<0.01			
Elevated total cholesterol, %	43.5	24.9	<0.01			
Triglycerides, mean (SD)	115 (83.4)	113 (77.2)	0.48			
Elevated triglycerides, %	19.8	19.7	0.91			
BMI, mean (SD)	23.9 (3.3)	23.0 (3.1)	<0.01			
BMI ≥24, %	45.7	33.5	<0.01			
Serum creatinine, mean (SD)	0.71 (0.22)	0.63 (0.23)	<0.01			
eGFR, mean (SD)	103 (15.1)	104.7 (13.8)	<0.01			
eGFR <60, %	1.5	1.1	0.26			

Abbreviations: BMI = body mass index; DBP = diastolic blood pressure; eGFR = estimated glomerular filtration rate; HDL = high-density lipoprotein cholesterol; LDL = low-density lipoprotein cholesterol; SBP = systolic blood pressure.
Elevated blood pressure is defined as SBP ≥120 or DBP ≥80 mm Hg, or both. Impaired fasting glucose is defined as ≥100 mg/dL. Elevated total cholesterol is defined as ≥200 mg/dL. Elevated triglycerides is defined as ≥150 mg/dL.

When examining specific stroke subtypes, vegetarians had 74% lower ischemic stroke risk throughout different models. Individuals with diabetes had about 3-fold risk of ischemic stroke when compared with nondiabetic patients (table e-2, doi.org/10.5061/dryad.x95x69pcz). In the sensitivity analysis, the protective association between vegetarian diet and ischemic stroke slightly attenuated after adjusting for fasting glucose, SBP, triglycerides, and total cholesterol (HR, 0.28; 0.08–0.96). Analysis of hemorrhagic stroke was not performed due to a small number of events (n = 8) in this cohort.

Table 2 Dietary intakes of vegetarians and nonvegetarians based on food frequency questionnaires in cohort 1 (Tzu Chi Health Study)

Foods and nutrients	Nonvegetarians (n = 3,626), median (IQR)	Vegetarians (n = 1,424), median (IQR)	p Value
Meat, servings	0.6 (0.2, 1.6)	—	—
Fish, servings	0.5 (0.1, 1.1)	—	—
Eggs, servings	0.3 (0.1, 0.6)	0.3 (0.1, 0.5)	<0.01
Dairy, servings	0.3 (0, 0.9)	0.2 (0, 0.7)	<0.01
Soy, servings	1.0 (0.5, 1.7)	1.5 (0.8, 2.5)	<0.01
Vegetables, servings	3.8 (2.3, 5.7)	4.5 (2.9, 6.8)	<0.01
Fruits, servings	1.0 (0.5, 2.0)	1.0 (0.6, 2.0)	0.07
Nuts, servings	0.2 (0.0, 0.5)	0.3 (0.1, 1.0)	<0.01
Energy, kcal	1740 (1,306, 2,297)	1705 (1,288, 2,247)	0.11
Protein (% energy)	13 (12, 15)	12 (11, 13)	<0.01
Animal protein, g	19 (11, 31)	4 (2, 7)	<0.01
Plant protein, g	38 (28, 51)	46 (34, 61)	<0.01
Fat (% energy)	27 (22, 33)	25 (19, 30)	<0.01
Saturate fat, g	11 (7, 17)	9 (6, 13)	<0.01
Monounsaturated fat, g	16 (10, 25)	12 (8, 19)	<0.01
Polyunsaturated fat, g	12 (7, 20)	11 (6, 18)	<0.01
Carbohydrate (% energy)	60 (53, 67)	64 (58, 70)	<0.01
Dietary fiber, g	19 (14, 26)	23 (16, 30)	<0.01
Vitamin B_{12}, μg	4.0 (1.9, 9.5)	1.4 (0.6, 5.2)	<0.01
Vitamin B_{12} intake <2.4 μg, %	33.3	64.0	<0.01
Use of vitamin B_{12} supplement, %	36.8	40.0	0.04

Abbreviation: IQR = interquartile range.
A serving of meat, fish, eggs, or soy is defined as 7 g protein; a serving of dairy is defined as 8 g protein; a serving of vegetable is defined as 100 g; a serving of fruit is defined as 15 g carbohydrates; a serving of nuts is defined as 45 kcal.

Table 3 Serum homocysteine, vitamin B_{12}, and folate concentration in 877 nonvegetarians and 406 vegetarians in cohort 1 (Tzu Chi Health Study)

	Nonvegetarians	Vegetarians	p Value
Homocysteine, μmol/L			
Median (IQR)	9.3 (7.9, 11.3)	10.6 (8.7, 14.0)	<0.01[a]
<12, %	82	63	<0.01[b]
≥12, <14, %	10	12	
≥14, ≤20, %	6	20	
>20, %	2	5	
Vitamin B_{12}, pg/mL			
Median (IQR)	487 (374, 653)	280 (196, 407)	<0.01[a]
<200, %	1	26	<0.01[b]
≥200, <300, %	10	28	
≥300, %	89	46	
Folate, ng/mL			
Median (IQR)	10.6 (7.9, 14.0)	13.4 (10.1, 17.4)	<0.01[a]

Abbreviation: IQR = interquartile range.
[a] p Value calculated using Wilcoxon rank-sum test.
[b] p Value calculated using χ^2 test.

For ischemic stroke, vegetarians consistently had about 60% lower risk than nonvegetarians throughout all models. Ever smoking (vs never) appeared to be independently associated with a nearly 2.5 times risk (table e-3, doi.org/10.5061/dryad.x95x69pcz). Having hypertension (vs none) was also associated with greater than 2-fold risk of ischemic stroke (table e-3, doi.org/10.5061/dryad.x95x69pcz).

For hemorrhagic stroke, vegetarians also experienced about 65% lower risk than nonvegetarians, and this finding appeared consistent throughout all models (table 4).

Subgroup analysis by vitamin B_{12} intakes

Our subgroup analysis by vitamin B_{12} intake is shown in table 5. Inverse association between vegetarian diet and lower overall stroke was found in the subgroup with inadequate vitamin B_{12} intake (<2.4 μg) but not in those with adequate vitamin B_{12} intake (≥2.4 μg), p interaction = 0.046. Baseline characteristic and dietary intakes of the subgroups as assessed by FFQ are presented in tables e-4 and e-5 (doi.org/10.5061/dryad.x95x69pcz), respectively. Vegetarians with adequate vitamin B_{12} intake consumed more animal protein (median 5 vs 3 g) and were more likely to take vitamin B_{12} supplements (96% vs 8.5%) than those with inadequate vitamin B_{12} intake.

Association between vegetarian diet and stroke incidence in cohort 2

There were 121 incident stroke events in the 76,797 person-years of follow-up. Stroke incidence in vegetarians was lower than in nonvegetarians (0.95 [95% CI, 0.64–1.42] vs 1.88 [95% CI, 1.54–2.29] per 1,000 person-years). Compared with nonvegetarians, vegetarians experienced only about half of the risk of overall stroke through all the models considered, as shown in table 4. Hypertension is an independent risk factor (HR, 1.82; 95% CI, 1.17–2.85), as shown in table e-3 (doi.org/10.5061/dryad.x95x69pcz).

Table 4 Association between vegetarian diet (vs nonvegetarian diet) and stroke incidence in cohort 1 (Tzu Chi Health Study) and cohort 2 (Tzu Chi Vegetarian Study)

	Nonvegetarian, cases/n	Vegetarian, cases/n	Crude model	Sex-adjusted	Main model	Final model	Sensitivity analysis
Cohort 1							
Overall stroke	44/3,626	10/1,424	0.50 (0.25, 1.01)	0.53 (0.26, 1.06)	0.55 (0.27, 1.11)	0.51 (0.25, 1.06)	0.54 (0.26, 1.13)
Ischemic stroke	28/3,626	3/1,424	0.25 (0.08, 0.83)	0.26 (0.08, 0.86)	0.26 (0.08, 0.88)	0.26 (0.08, 0.88)	0.28 (0.08, 0.96)
Cohort 2							
Overall stroke	97/5,583	24/2,719	0.47 (0.30, 0.74)	0.50 (0.32, 0.78)	0.51 (0.33, 0.80)	0.52 (0.33, 0.82)	
Ischemic stroke	46/5,583	8/2,719	0.33 (0.16, 0.70)	0.37 (0.17, 0.78)	0.39 (0.18, 0.84)	0.41 (0.19, 0.88)	
Hemorrhagic stroke	24/5,583	4/2,719	0.32 (0.11, 0.91)	0.34 (0.12, 1.00)	0.35 (0.12, 1.02)	0.34 (0.12, 1.00)	

Values are hazard ratios (95% confidence intervals) estimated by Cox regression with age as time scale. Sex-adjusted model adjusted for sex only. Main model adjusted for sex, smoking (ever vs never), alcohol drinking (ever vs never), betel nut (ever vs never), leisure time physical activities (yes vs no), and education. Final model adjusted for all variables in main model, plus hypertension, diabetes mellitus, dyslipidemia, ischemic heart disease, and body mass index (≥24 vs <24, for cohort 1 only). Sensitivity analysis adjusted for all variables in main model, plus body mass index (≥24 vs <24), systolic blood pressures, fasting glucose, total cholesterol, and triglycerides.

Discussion

Our prospective cohort studies showed that a vegetarian diet is associated with a lower risk of both ischemic stroke and hemorrhagic stroke. Potential effect modification by dietary vitamin B_{12} intake was found for overall stroke.

The most important modifiable risk factor for stroke is hypertension, accounting for 47.9%, 45.7%, and 56.4% of population attributable risk for overall, ischemic, and hemorrhagic strokes, respectively, in the INTERSTROKE study.[4] In our cohort studies, vegetarians had lower baseline prevalence of elevated BPs (23 vs 30% in cohort 1) and hypertension (9.4 vs 11.5% in cohort 2) than nonvegetarians. The inverse association between vegetarian diet and BPs in our study is consistent with a previous systematic review and meta-analysis[8] and a prospective study.[24] The health benefits of a plant-based diet for BP control have been so well-documented that the

Dietary Approach to Stop Hypertension (DASH)—the gold standard diet for hypertension management—emphasizes increased consumption of healthy plant-based food and minimization of red and processed meat, and this diet has also been shown to lower the risk for overall stroke[12] and ischemic stroke.[13] The PREDIMED trial that randomized 7,447 high-risk participants into the Mediterranean diet (also a plant-based pattern) vs control diet found that the Mediterranean diet reduced the risk of overall stroke by 42% compared with the control diet, supporting the consistent beneficial effect of plant-based dietary patterns.[11] Since the incidence of ischemic stroke is much higher than that of the hemorrhagic type globally and in Taiwan, the protective association between diet and overall stroke may be attributable to ischemic stroke.

In addition to hypertension, vegetarian diets have been shown to lower other important risk factors of ischemic stroke, including both low-density lipoprotein (LDL)–cholesterol and

Table 5 Subgroup analysis by vitamin B_{12} intake on the association between vegetarian diet (vs nonvegetarian diet) and overall stroke incidence in cohort 1 (Tzu Chi Vegetarian Study)

	Nonvegetarians, cases/n	Vegetarians, cases/n	HR (95% CI)	p interaction
Vitamin B_{12} intake <2.4 μg/d	20/1,208	4/911	0.27 (0.09, 0.83)	0.046
Vitamin B_{12} intake ≥2.4 μg/d	24/2,418	6/513	0.99 (0.38, 2.57)	

Abbreviations: CI = confidence interval; HR = hazard ratio.
HR is estimated using Cox regression, with age as time scale, and adjustment for sex (men vs women), smoking (ever vs never), alcohol drinking (ever vs never), betel nut (ever vs never), leisure time physical activities (yes vs no), education (elementary or less vs secondary vs college or higher), hypertension (yes vs no), diabetes (yes vs no), dyslipidemia (yes vs no), ischemic heart diseases (yes vs no), body mass index (≥24 vs <24), systolic blood pressures, fasting glucose, total cholesterol, and triglycerides.

diabetes.[6,25] The mean concentration of LDL-cholesterol in our vegetarians was 11% lower than in the nonvegetarians (115 vs 129 mg/dL). This may contribute to significant protection, as a 10% reduction in LDL-cholesterol has been shown to reduce the relative risk of primary stroke by 13.5% in a meta-analysis of statin trials.[26] Vegetarian dietary pattern in our population is characterized by higher intake of vegetables, soy, and nuts. These plant foods are major sources of antioxidants,[27] which could reduce oxidative stress known to impair endothelial function and induce atherosclerosis.[28] Higher consumption of plant protein, dietary fiber, and antioxidants have all been shown to protect against stroke in prospective studies.[29–31]

In our study, the beneficial association between vegetarian diet and ischemic stroke was attenuated only slightly after controlling for known cardiometabolic mediators (BPs, glucose, and lipid) or comorbidities (hypertension, diabetes, dyslipidemia, and ischemic heart diseases). This suggests that additional protective mechanisms may exist. Carnitine from meat and choline from eggs not only provide substrates for but also shift gut microbial community to enhance the production of trimethylamine N-oxide (TMAO),[32] an atherosclerotic factor that has been shown to induce platelet hyperreactivity and thrombosis[33] and increase stroke incidence.[34] Vegetarians' chance of being a high TMAO producer is only one-tenth that of nonvegetarians,[35] and this may exert additional protection on top of the traditional risk factors for stroke.

The strong inverse association between vegetarian diet and hemorrhagic stroke in our study contrasts the results of the recently published EPIC-Oxford study.[36] Buddhist vegetarians in our population and the EPIC-Oxford vegetarians differ in alcohol consumption and use of allium vegetables. Nearly 80% of vegetarians in the EPIC-Oxford study drink varying degrees of alcohol but most participants in our studies avoid alcohol (about 6% of nonvegetarians and fewer than 1% of vegetarians were current drinkers at baseline). Alcohol consumption (as measured by γ-glutamyl transferase) has been suggested in a previous cohort study to modify the effect of low serum cholesterol (typical of vegetarians) on hemorrhagic stroke risk.[37] The EPIC-CVD case cohort study also found a clear trend between increased alcohol consumption and stroke risk.[38] While Western vegetarians use ample amounts of garlic and other allium vegetables, Asian Buddhist vegetarians avoid these vegetables. Allium vegetables are major sources of alliin/allicin, known to inhibit platelet function and induce bleeding.[39] Whether these differences drive the discrepant findings warrants further investigation.

Hypertension is the most important risk factor for hemorrhagic stroke.[4] Vegetarian diet and other plant-based diets such as DASH and Mediterranean dietary patterns effectively reduce BPs, as evidenced by systematic review and meta-analysis of randomized controlled trials.[8,40] Previous cohort studies found that the highest vs lowest adherence to DASH and Mediterranean diet were associated with a 10%—20% reduction in hemorrhagic stroke.[12,14,41] In addition to the BP-lowering

effect common to most plant-based diets, a vegetarian diet may have the additional advantage of reduced TMAO,[35] and TMAO has been positively associated with hemorrhagic stroke risk among hypertensive individuals in a recent nested case–control study.[34]

Many studies reporting inverse associations between intake of animal products and hemorrhagic stroke focus on a single food or nutrient.[16,29,42] The effect of animal products or nutrients would be difficult to single out if they correlate highly with other protective plant foods. This is not uncommon in Asian cultures: in the Japanese Public Health Center–based prospective study, fish appears to be a marker for a healthy dietary pattern that simultaneously contains higher vegetables, fruits, soy, seaweeds, potato, and mushroom, derived from principal component analysis.[43] The dietary cluster with the lowest fish and meat intake also contained the lowest vegetables and fruits in the Shanghai Women's Health Study.[44]

Despite higher homocysteine due to lower vitamin B_{12} intake, vegetarians did not have higher risk of overall, ischemic, or hemorrhagic strokes. A recent Mendelian randomization analysis and the Vitamins to Prevent Stroke (VITATOPS) trial both found that lowering of homocysteine is associated with only the small vessel subtype of ischemic stroke—which accounts for 37.7% of ischemic and 27.9% of overall stroke in Taiwan[2]—but not other subtypes (large artery or cardioembolic).[41,45] The ICD-9-CM code could not distinguish to such detail and our studies would not have enough power to examine these detailed subtypes of ischemic stroke. In our study, the percentage of study participants with hyperhomocysteinemia (defined as >20 μmol/L) is low (2% in nonvegetarians vs 5% in vegetarians), likely due to healthy overall lifestyle (nonsmoking, no alcohol drinking, adequate folate intake through high vegetable consumption), thus stroke risk attributable to hyperhomocysteinemia is probably low.

Interestingly, our study found that the inverse association between vegetarian diet and overall stroke risk is more pronounced in the subgroup consuming less than the RDA of vitamin B_{12}, but not the subgroup with adequate intakes (p interaction = 0.046). Supplementation with high-dose cyanocobalamin (a form of vitamin B_{12}) increases the risk of stroke among individuals with reduced renal function.[46] Although we have no data on the type of vitamin B_{12} supplements (cyanocobalamin, methylcobalamin, or hydroxocobalamin) to clarify whether the attenuation of protection is related to cyanocobalamin, it is unlikely to be the major reason as the percent of vegetarians with abnormal renal function is small (1.8% in vegetarians meeting the RDA for vitamin B_{12}). Vitamin B_{12} may be a surrogate marker for animal product consumption and this may account for some attenuation. Due to small sample size, this finding needs to be interpreted with caution and confirmed in other studies.

Our study has several strengths. First, the high proportion of vegetarians (~30%) enables us to examine the association

between vegetarian diet and stroke risk with adequate power. Second, the high follow-up rate due to nearly complete coverage in the NHIRD prevents loss to follow-up and minimizes selection bias. Third, data on stroke-relevant metabolic risk factors, such as fasting blood glucose, total cholesterol, BPs, and BMI, were available. Fourth, the consistent results in both cohorts strengthen confidence in the finding. Finally, vegetarians and nonvegetarians were from the same religious group and abstain from alcohol and tobacco; this would minimize confounding associated with religion and related lifestyle, and strengthen internal validity, while providing a unique opportunity to examine the effect of plant-based diet among people who do not drink alcohol, which is rare in most Western studies.

Several limitations should be considered when interpreting these findings. First, diet was assessed only at baseline and change over time is possible, though this should attenuate the protective effect of the vegetarian diet—bias toward the null. Second, the definition of stroke is based on ICD-9-CM, not MRI findings, and there are no data on detailed subtypes within ischemic stroke or hemorrhagic stroke. However, the stroke ICD-9-CM codes in NHIRD have been validated and demonstrated a 97% positive predictive value with specified coding.[20] Third, the generalizability of study findings may be limited to populations that abstain from cigarettes and alcohol. The overall finding contrasts with that of the EPIC-Oxford study but is consistent with previous studies on plant-based diets and stroke.[11–14] Fourth, the small sample size made it difficult to evaluate the association between the vegetarian diet and stroke subtypes, or make conclusion on subgroup analysis and interaction. Finally, residual confounding may be present, despite extensive adjustments for potential confounding factors, due to the observational study design.

Author contributions

T.H.T.C., H.-R.C., and L.-Y.W. wrote the paper. L.-Y.W. and C.-C.C. conducted the statistical analysis. M.-N.L. and C.-L.L. designed the study and collected the baseline data.

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Disclosure

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