Lack of association between dietary protein intake and risk of stroke among middle-aged men[1–3]

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ABSTRACT

Background: Prospective cohort studies in Japanese populations have shown an inverse association between dietary protein and stroke risk. However, this association has not been examined among any study populations of US men.

Objective: Our objective was to examine the relation between dietary protein and risk of stroke in men who participated in the Health Professionals Follow-Up Study.

Design: A total of 43,960 men who were free of cardiovascular disease and cancer at baseline were included in the analysis. Dietary protein (total, animal, and vegetable) was assessed with the use of a food-frequency questionnaire at 5 time points during the follow-up period of 1986–2004. Cox proportional hazards models were used to calculate multivariate relative risks and 95% CIs, which represented the effect of the substitution of protein for an equal percentage of energy from carbohydrate.

Results: During 18 y of follow-up there were 1057 incident stroke events (638 ischemic, 171 hemorrhagic, and 248 of unknown type). For total stroke, the relative risk for the top quintile of percentage energy from protein compared with the bottom was 1.14 (95% CI: 0.90, 1.43; *P* for linear trend: 0.43) for total protein, 1.11 (95% CI: 0.87, 1.41; *P* for linear trend: 0.52) for animal protein, and 0.82 (95% CI: 0.60, 1.12; *P* for linear trend: 0.17) for vegetable protein. The results were similar when ischemic and hemorrhagic stroke subtypes were considered separately.

Conclusion: In contrast to studies in Japanese populations, this study did not show a statistically significant association between total, animal, or vegetable protein and risk of stroke in this population of US men. *Am J Clin Nutr* 2010;91:39–45.

INTRODUCTION

Several studies in Japanese populations have suggested that dietary protein, especially protein from animal sources, may decrease the risk of stroke (1–3). In observational studies, as well as in clinical trials, dietary protein intake was inversely associated with blood pressure (4). Because hypertension is a strong risk factor for stroke, it is plausible that a higher intake of dietary protein may decrease the risk of stroke.

Because the Japanese diet is markedly different from the Western diet, especially with respect to protein and fat intake, it is possible that the relation between protein and stroke risk may be different in a US population (2). Additionally, Japanese studies have focused on intraparenchymal hemorrhage, which occurs more commonly in Japan relative to Western populations, and not on ischemic stroke, which is more prevalent in the United States and Europe. We therefore assessed the effect of the substitution of dietary protein for carbohydrate on the risk of total, ischemic, and hemorrhagic stroke in a group of male US health professionals over an 18-y follow-up period.

SUBJECTS AND METHODS

Study population

The Health Professionals Follow-Up Study, which began in 1986, is an ongoing, prospective cohort study of 51,529 men aged 40–75 y at baseline. Beginning in 1986, the cohort participants were sent a biennial questionnaire with questions about diseases and lifestyle characteristics. Every 4 y the cohort participants completed a food-frequency questionnaire (FFQ). At least one follow-up questionnaire has been completed by ≈94% of the cohort. We excluded those who reported a history of stroke, angina, coronary artery bypass graft, other heart conditions, myocardial infarction, pulmonary embolism, or cancer on the baseline questionnaire. Additionally, those who had an implausible caloric intake (<800 or >4200 kcal/d) or had >70 missing responses to food items were excluded, which resulted in a population of 43,960 for the current analysis. This study was approved by the Harvard Institutional Review Board.

Dietary assessment

Dietary intake was measured by a 131-item FFQ, which was mailed to the participants at baseline (1986) and in 1990, 1994, 1998, and 2002. Details of the assessment of nutrient values have been described previously (5). Percentage energy from protein intake was calculated by multiplying the grams of protein consumed per day by the number of kilocalories in one gram of

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[2] Supported by grants from the National Institutes of Health (HL35464 and CA55075) and by the Kirschstein-National Research Service Award (NRSA) Aging Training Grant (AG000158).

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Received May 11, 2009. Accepted for publication October 6, 2009.

First published online November 4, 2009; doi: 10.3945/ajcn.2009.28060.

protein (4 kcal/g) and then dividing by the subject's total caloric intake (6). Carbohydrates and dietary fats (saturated, monounsaturated, polyunsaturated, and *trans*) were also expressed as a percentage of energy, with the assumption of 4 kcal/g of carbohydrate and 9 kcal/g of fat. All other nutrients, with the exception of alcohol, were energy adjusted with the use of the residual method (7).

A subsample of the baseline study population was used to evaluate the validity of the 131-item FFQ (8). The de-attenuated (9), energy-adjusted Pearson correlation coefficient for the macronutrients between the average of the diet records and the FFQ was 0.67 for fat, 0.73 for carbohydrate, and 0.44 for protein.

Assessment of stroke endpoints

The primary endpoints of interest were incident ischemic and hemorrhagic stroke that occurred between the return of the 1986 questionnaire and 31 January 2004. Nonfatal stroke was assessed biennially with the use of a mailed questionnaire that contained questions that related to medical conditions that occurred in the time period after the prior questionnaire. A physician verified the report of incident stroke through review of medical/hospital records that included neuroimaging (computerized tomography/magnetic resonance imaging) results. All strokes were classified as ischemic (thrombotic or embolic), hemorrhagic [subarachnoid or intracerebral (intraparenchymal)], or of unknown type (when the physician could not determine the classification), in accordance with the criteria defined in the National Survey of Stroke (10).

We ascertained deaths by contact with family members and by a search of the National Death Index. Fatal strokes were confirmed from medical records or autopsy reports. Strokes were considered probable if medical records or autopsy reports could not be obtained but stroke was listed as the underlying cause of death on the death certificate.

Statistical analysis

A multivariate nutrient density model was used to analyze the association between protein intake and risk of stroke (7, 11). We constructed age-adjusted nutrient density models, which included quintiles of percentage energy from protein, saturated fat, monounsaturated fat, polyunsaturated fat, and *trans* fat and total energy intake. We also constructed a multivariate nutrient density model that contained all variables in the age-adjusted model plus the following covariates: body mass index (BMI; in kg/m^2) ($<$23, 23–24.9, 25–28.9, $\geq$29), cigarette smoking (never smoked cigarettes; nonsmoker of cigarettes with unknown past history; past smoker; current smoker of 1–14, 15–24, $\geq$25 cigarettes/d; current smoker but unknown number of cigarettes/d), parental history of myocardial infarction before age 65 (yes, no), alcohol consumption (0, 0.1–4.9, 5–14.9, $\geq$15 g/d), multivitamin use (yes, no), and quintiles of physical activity [metabolic equivalent tasks (METS)/d], glycemic index, folate (μg/d), fiber (g/d), vitamin B-6 (mg/d), vitamin B-12 (μg/d), vitamin C (mg/d), potassium (mg/d), magnesium (mg/d), and total omega-3 fatty acids (g/d). We also constructed a model that contained baseline (1986) status of hypertension, diabetes, and hypercholesterolemia, in addition to the variables in the multivariate nutrient density model, because participants who developed hypertension, diabetes, or hypercholesterolemia before the start of the study may have altered their diet after those diagnoses. Assessment of hypertension, diabetes, or hypercholesterolemia was based on self-report of a physician diagnosis of the condition on the participant's biennial questionnaire. The variables for hypertension, diabetes, and hypercholesterolemia occurrence during the study were not controlled for because these conditions are potential intermediates on the causal pathway between dietary protein intake and stroke (12). The coefficient for protein in the nutrient density model can be interpreted as the effect of the substitution of an equal amount of energy from protein for carbohydrate.

With the repeated measurements of dietary protein, we used the cumulative average approach to assign an individual's intake at each time period, which gives greater weight to more recent diet (11). This approach has been shown to minimize measurement error because it incorporates all prior dietary assessments taken during follow-up (11). If a person developed an intermediate event that may have altered their diet (hypercholesterolemia, hypertension, angina, diabetes, cancer), only their diet before the diagnosis of that condition was considered in the analysis.

Each participant contributed person-time to the analysis, starting from the date of the return of their 1986 questionnaire until 31 January 2004, death, loss to follow-up, diagnosis of cancer or ischemic heart disease, or development of stroke, whichever occurred first. Incidence rates of stroke for each quintile of percentage of energy from protein were calculated by dividing the number of cases by the total person-time at risk. Relative risks (RRs) and 95% CIs were calculated with the use of a Cox proportional hazards regression model that was stratified jointly by age in months and the 8 time periods that corresponded to the follow-up questionnaire cycles, with the use of PROC PHREG in SAS, version 9.1 (SAS Institute Inc, Cary, NC).

To test for linear trends we used the median protein intake of each quintile as a quantitative single variable and assessed the significance of this term by using a 1 df Wald test. The interaction of protein intake with the traditional cardiovascular disease (CVD) risk factors was examined by the inclusion of a cross-product term for the median score variable for protein multiplied by the risk factor. The significance of the interaction terms was assessed with the use of the likelihood ratio test statistic. The risk factors considered were hypertension (yes, no), hypercholesterolemia (yes, no), diabetes (yes, no), BMI ($\geq$25, $<$25), and glycemic index ($<$55, $\geq$55).

RESULTS

During the 18 y (682,568 person-years) of follow-up, among the 43,960 participants included in the analysis, we documented 1057 incident stroke events (638 ischemic, 171 hemorrhagic, and 248 of unknown type). Baseline characteristics of the study population according to quintile of percentage energy from total protein are presented in **Table 1**.

Total protein and stroke risk

When those in the top total protein quintile (median = 22.5% of energy) were compared with those in the bottom quintile (median = 14.6% of energy), with adjustments for age, total energy intake,

TABLE 1
Distribution of stroke risk factors by quintile of percentage energy from total protein at baseline (1986) among participants in the Health Professionals Follow-Up Study, 1986–2004 (n = 43,960)[1]

	Quintile 1	Quintile 3	Quintile 5	P for linear trend[2]
Age (y)	53 ± 10[3]	53 ± 9	55 ± 9	<0.001
Cigarette smoking status [n (%)]				
Never	3991 (45.3)	4000 (47.4)	4430 (45.0)	(Referent)
Past	3402 (38.6)	3337 (39.6)	4134 (42.0)	0.009
1–14 cigarettes/d	261 (3.0)	215 (2.6)	251 (2.6)	0.07
15–24 cigarettes/d	334 (3.8)	294 (3.5)	276 (2.8)	<0.001
≥25 cigarettes/d	390 (4.4)	215 (2.6)	210 (2.1)	<0.001
Unknown no. of cigarettes/d	105 (1.2)	73 (0.9)	96 (1.0)	0.14
Missing	337 (3.8)	302 (3.6)	447 (4.5)	0.03
History of hypertension [n (%)]	1819 (20.6)	1810 (21.5)	2516 (25.6)	<0.001
History of hypercholesterolemia [n (%)]	807 (9.2)	872 (10.3)	1228 (12.5)	<0.001
History of diabetes [n (%)]	114 (1.3)	148 (1.8)	507 (5.2)	<0.001
Parental history of myocardial infarction <65 y [n (%)]	1025 (11.6)	974 (11.6)	1287 (13.1)	<0.001
Exercise (METS)	20.9 ± 30.0	20.8 ± 27.4	21.1 ± 29.1	0.88
BMI (kg/m^2)	25.1 ± 3.2	25.5 ± 3.4	25.9 ± 3.6	<0.001
Nutrients				
Calories (kcal/d)	2137 ± 664	2025 ± 598	1800 ± 574	<0.001
Total protein (% of energy)	14.2 ± 1.4	18.2 ± 0.4	23.2 ± 2.3	<0.001
Animal protein (% of energy)	9.3 ± 1.8	13.2 ± 1.2	18.3 ± 2.7	<0.001
Vegetable protein (% of energy)	4.9 ± 1.3	5.0 ± 1.2	4.9 ± 1.3	0.60
Carbohydrates (% of energy)	50.9 ± 9.3	46.9 ± 7.5	42.9 ± 7.9	<0.001
Saturated fat (% of energy)	10.5 ± 2.8	11.2 ± 2.7	11.1 ± 2.9	<0.001
Monounsaturated fat (% of energy)	11.8 ± 2.8	12.5 ± 2.6	12.2 ± 2.8	<0.001
Polyunsaturated fat (% of energy)	5.9 ± 1.8	6.0 ± 1.5	6.0 ± 1.5	<0.001
trans Fat (% of energy)	1.4 ± 0.6	1.3 ± 0.5	1.1 ± 0.4	<0.001
Alcohol (% of energy)	5.9 ± 7.4	3.9 ± 4.8	2.7 ± 3.6	<0.001
Alcohol (g/d)	17.5 ± 22.0	11.0 ± 13.5	6.8 ± 9.1	<0.001
Folate, energy-adjusted (μg/d)	442 ± 254	476 ± 262	519 ± 312	<0.001
Vitamin B-6, energy-adjusted (mg/d)	7.1 ± 21.4	8.0 ± 23.1	11.4 ± 31.7	<0.001
Vitamin B-12, energy-adjusted (μg/d)	10.1 ± 11.9	12.2 ± 12.2	15.3 ± 24.8	<0.001
Potassium, energy-adjusted (mg/d)	3088 ± 709	3426 ± 644	3727 ± 742	<0.001
Fiber, energy-adjusted (g/d)	19.9 ± 7.6	21.0 ± 6.6	21.6 ± 7.5	<0.001
Vitamin C, energy-adjusted (mg/d)	389 ± 423	421 ± 468	488 ± 531	<0.001
Magnesium, energy-adjusted (mg/d)	324 ± 81	351 ± 76	382 ± 92	<0.001
Omega-3 fatty acids, energy-adjusted (g/d)	1.3 ± 0.4	1.4 ± 0.4	1.6 ± 0.5	<0.001
Glycemic index	54.0 ± 3.8	53.3 ± 3.3	52.0 ± 3.9	<0.001

[1] METS, metabolic equivalent tasks.
[2] Across quintiles 1 through 5.
[3] Mean ± SD (all such values).

and the other macronutrients, the RR for total stroke was 1.02 (95% CI: 0.84, 1.24) (**Table 2**). This model has the interpretation of the substitution of protein for an isocaloric amount of carbohydrate. Further adjustment for additional dietary variables and CVD risk factors resulted in an RR of 1.25 (95% CI: 0.99, 1.57) for total stroke. After additional adjustment for baseline status of hypertension, hypercholesterolemia, and diabetes, the RR decreased to 1.14 (95% CI: 0.90, 1.43; P for linear trend: 0.43). The RRs comparing extreme quintiles of total protein for ischemic stroke and hemorrhagic stroke separately were very similar to those for total stroke.

Animal protein and stroke risk

The Cox proportional hazards model results for the association between animal protein and total stroke were similar to those for total protein. In multivariate models that controlled for age, macronutrients, and total energy intake, the RR for those in the top quintile of animal protein intake (median = 17.7% of energy) compared with those in the bottom (median = 9.3% of energy) was 1.01 (95% CI: 0.82, 1.24). In the fully adjusted model, the RR for the top quintile was 1.11 (95% CI: 0.87, 1.41; P for linear trend: 0.52). The results for ischemic stroke and hemorrhagic stroke were comparable to those for total stroke.

Vegetable protein and stroke risk

For vegetable protein we found an inverse association for total stroke and ischemic stroke in the age- and macronutrient-adjusted RR when those in the top quintile (median = 6.5% of energy) were compared with those in the bottom quintile (median = 3.7% of energy). For total stroke the RR was 0.72 (95% CI: 0.57, 0.90; P for linear trend: 0.002) and for ischemic stroke the RR was 0.64 (95% CI: 0.48, 0.86; P for linear trend: 0.005). However, in the fully adjusted model, the RRs for both total and ischemic stroke were no longer statistically significant. There was no association between vegetable protein risk of hemorrhagic

TABLE 2
Relative risks and 95% CIs of stroke by quintile of percentage energy from protein among participants in the Health Professionals Follow-Up Study, 1986–2004 (n = 43,960)[1]

	Quintile 1	Quintile 2	Quintile 3	Quintile 4	Quintile 5	P for linear trend
Total protein						
Median (% of energy)	14.6	16.7	18.2	19.8	22.5	
Total stroke (no. of cases)	223	201	208	186	239	
Model 1	1.00 (referent)	0.93 (0.76, 1.13)	0.97 (0.80, 1.18)	0.81 (0.66, 0.99)	1.02 (0.84, 1.24)	0.86
Model 2	1.00 (referent)	1.02 (0.83, 1.24)	1.12 (0.91, 1.38)	0.97 (0.77, 1.20)	1.25 (0.99, 1.57)	0.09
Model 3	1.00 (referent)	1.01 (0.83, 1.24)	1.08 (0.88, 1.33)	0.92 (0.74, 1.14)	1.14 (0.90, 1.43)	0.43
Ischemic stroke (no. of cases)	134	126	123	111	144	
Model 1	1.00 (referent)	0.94 (0.73, 1.21)	0.93 (0.72, 1.19)	0.77 (0.60, 1.00)	0.97 (0.75, 1.24)	0.52
Model 2	1.00 (referent)	1.04 (0.80, 1.34)	1.10 (0.84, 1.43)	0.94 (0.71, 1.25)	1.24 (0.92, 1.66)	0.25
Model 3	1.00 (referent)	1.02 (0.79, 1.32)	1.07 (0.82, 1.40)	0.90 (0.67, 1.19)	1.13 (0.84, 1.52)	0.61
Hemorrhagic stroke (no. of cases)	37	37	33	29	35	
Model 1	1.00 (referent)	1.59 (0.90, 2.79)	1.34 (0.75, 2.40)	1.22 (0.67, 2.20)	1.12 (0.60, 2.08)	0.93
Model 2	1.00 (referent)	1.84 (1.01, 3.33)	1.59 (0.85, 2.97)	1.53 (0.79, 2.96)	1.36 (0.66, 2.81)	0.64
Model 3	1.00 (referent)	1.85 (1.02, 3.36)	1.56 (0.83, 2.91)	1 47 (0.76, 2.85)	1.24 (0.59, 2.58)	0.85
Animal protein[2]						
Median (% of energy)	9.3	11.5	13.1	14.8	17.7	
Total stroke (no. of cases)	204	208	204	207	234	
Model 1	1.00 (referent)	1.05 (0.86, 1.28)	0.99 (0.81, 1.22)	0.99 (0.80, 1.21)	1.01 (0.82, 1.24)	0.88
Model 2	1.00 (referent)	1.11 (0.90, 1.36)	1.11 (0.89, 1.37)	1.13 (0.91, 1.41)	1.22 (0.96, 1.55)	0.13
Model 3	1.00 (referent)	1.10 (0.89, 1.34)	1.08 (0.87, 1.33)	1.06 (0.85, 1.32)	1.11 (0.87, 1.41)	0.52
Ischemic stroke (no. of cases)	127	118	126	126	141	
Model 1	1.00 (referent)	0.92 (0.71, 1.19)	0.92 (0.71, 1.19)	0.91 (0.70, 1.18)	0.90 (0.68, 1.17)	0.46
Model 2	1.00 (referent)	0.97 (0.74, 1.26)	1.04 (0.79, 1.36)	1.06 (0.80, 1.41)	1.12 (0.82, 1.52)	0.39
Model 3	1.00 (referent)	0.95 (0.73, 1.24)	1.02 (0.78, 1.33)	1.00 (0.75, 1.32)	1.03 (0.75, 1.40)	0.80
Hemorrhagic stroke (no. of cases)	33	42	31	32	33	
Model 1	1.00 (referent)	1.58 (0.90, 2.77)	1.00 (0.54, 1.84)	1.18 (0.64, 2.16)	1.13 (0.60, 2.13)	0.90
Model 2	1.00 (referent)	1.83 (1.02, 3.28)	1.23 (0.64, 2.34)	1.54 (0.79, 2.97)	1.41 (0.67, 2.94)	0.58
Model 3	1.00 (referent)	1.83 (1.02, 3.29)	1.19 (0.62, 2.27)	1.45 (0.75, 2.82)	1.26 (0.60, 2.65)	0.83
Vegetable protein[3]						
Median (% of energy)	3.7	4.4	4.9	5.5	6.5	
Total stroke (no. of cases)	245	218	203	202	189	
Model 1	1.00 (referent)	0.92 (0.76, 1.12)	0.84 (0.69, 1.03)	0.79 (0.65, 0.98)	0.72 (0.57, 0.90)	0.002
Model 2	1.00 (referent)	1.02 (0.82, 1.25)	0.97 (0.76, 1.23)	0.93 (0.71, 1.21)	0.84 (0.61, 1.14)	0.21
Model 3	1.00 (referent)	1.02 (0.83, 1.26)	0.98 (0.78, 1.25)	0.93 (0.72, 1.21)	0.82 (0.60, 1.12)	0.17
Ischemic stroke (no. of cases)	157	128	113	131	109	
Model 1	1.00 (referent)	0.86 (0.67, 1.10)	0.74 (0.57, 0.96)	0.82 (0.63, 1.06)	0.64 (0.48, 0.86)	0.005
Model 2	1.00 (referent)	0.93 (0.71, 1.21)	0.84 (0.62, 1.14)	0.96 (0.69, 1.34)	0.79 (0.53, 1.19)	0.33
Model 3	1.00 (referent)	0.94 (0.72, 1.22)	0.86 (0.63, 1.18)	0.98 (0.70, 1.36)	0.80 (0.53, 1.19)	0.33
Hemorrhagic stroke (no. of cases)	32	36	43	28	32	
Model 1	1.00 (referent)	1.05 (0.59, 1.87)	1.30 (0.74, 2.30)	1.02 (0.55, 1.91)	1.06 (0.53, 2.09)	0.92
Model 2	1.00 (referent)	1.12 (0.59, 2.13)	1.42 (0.70, 2.86)	1.13 (0.51, 2.51)	1.16 (0.46, 2.95)	0.85
Model 3	1.00 (referent)	1.15 (0.61, 2.18)	1.46 (0.72, 2.95)	1.14 (0.52, 2.54)	1.16 (0.46, 2.95)	0.87

[1] Relative risks (95% CIs) were calculated with the use of Cox proportional hazards regression models. Model 1 was adjusted for age and quintiles of percentage of energy from saturated fat, monounsaturated fat, polyunsaturated fat, and *trans* fat and quintiles of calories. Model 2 was the same as Model 1 with additional adjustments for quintiles of fiber, folate, vitamin B-6, vitamin B-12, potassium, vitamin C, magnesium, total omega-3 fatty acids, glycemic index and physical activity, family history of myocardial infarction (yes, no), BMI (in kg/m^2; $<$23, 23–24.9, 25–28.9, $\geq$29), cigarette smoking (never smoked cigarettes; nonsmoker with unknown past history; past smoker; current smoker of 1–14, 15–24, $\geq$25 cigarettes/d; current but unknown no. of cigarettes/d), and alcohol (0, 0.1–4.9, 5–14.9, $\geq$15 g/d) and multivitamin use (yes, no). Model 3 was the same as Model 2 with additional adjustments for baseline (1986) status of hypertension, hypercholesterolemia, and diabetes.

[2] Additionally adjusted for quintiles of percentage of energy from vegetable protein.

[3] Additionally adjusted for quintiles of percentage of energy from animal protein.

stroke; the RR in the fully adjusted model was 1.16 (95% CI: 0.46, 2.95).

Dietary protein and risk of intraparenchymal hemorrhage

Because previous studies have shown a strong inverse association between animal protein and risk of intraparenchymal hemorrhage (1–3), we examined this relation by using the 128 intraparenchymal hemorrhage cases in our cohort. The multivariate RR in the comparison of extreme quintiles was 1.70 (95% CI: 0.72, 4.01) for total protein, 1.50 (95% CI: 0.62, 3.60) for animal protein, and 1.32 (95% CI: 0.44, 3.97) for vegetable protein.

Exclusion of participants with CVD risk factors at baseline

Our study population contained participants who had baseline conditions, namely hypertension, diabetes, and hypercholesterolemia, which may have led to a change in their diet before the onset of the study. The main results were attenuated when these potential confounders were controlled for (Table 2). However, to assess the presence of confounding, we also repeated the analysis with the exclusion of those who reported a diagnosis of hypertension, diabetes, or hypercholesterolemia at baseline. For total stroke (n = 556) we found that the results were similar to those for the full cohort. For example, the RRs in the comparison of extreme quintiles were 1.12 (95% CI: 0.82, 1.54; P for linear trend: 0.67) for total protein, 1.17 (95% CI: 0.83, 1.64; P for linear trend: 0.53) for animal protein, and 0.86 (95% CI: 0.55, 1.34; P for linear trend: 0.35) for vegetable protein. For ischemic stroke (n = 323) the RRs for total and animal protein were further from the null than those in the full cohort but the RR for vegetable protein was similar. The RRs for total, animal, and vegetable protein were 1.17 (95% CI: 0.77, 1.76; P for linear trend: 0.48), 1.25 (95% CI: 0.81, 1.93; P for linear trend: 0.30), and 0.76 (95% CI: 0.42, 1.36; P for linear trend: 0.40), respectively. For hemorrhagic stroke (n = 82) the RRs for total, animal, and vegetable protein were 1.22 (95% CI: 0.47, 3.15; P for linear trend: 0.85), 0.86 (95% CI: 0.32, 2.32; P for linear trend: 0.49), and 1.21 (95% CI: 0.32, 4.52; P for linear trend: 0.84), respectively.

Interactions between dietary protein and CVD risk factors

We further examined the interaction of total, animal, and vegetable protein with the following factors: hypertension, diabetes, hypercholesterolemia, high BMI ($\geq$25), and low average glycemic index ($<$55). We found a suggestion of an increased risk associated with higher total protein among men with hypercholesterolemia for both total and ischemic stroke. The RR for total stroke for the comparison of extreme quintiles of total protein was 1.38 (95% CI: 0.93, 2.03) among those with hypercholesterolemia and 1.02 (95% CI: 0.75, 1.38) among those without hypercholesterolemia ($P_{\text{interaction}}$ = 0.05). The RR for ischemic stroke for the comparison of extreme quintiles of total protein was 1.42 (95% CI: 0.83, 2.43) among those with hypercholesterolemia and 0.84 (95% CI: 0.54, 1.31) among those without hypercholesterolemia ($P_{\text{interaction}}$ = 0.07). We did not observe any statistically significant interactions by diabetes status, hypertension status, BMI, or glycemic index for total or ischemic stroke (data not shown). Because of the small number of cases of hemorrhagic stroke, we were not able to conduct any meaningful stratified analyses for that stroke subtype.

DISCUSSION

We examined the risk of stroke associated with the substitution of an equal percentage of energy from total, animal, and vegetable protein for carbohydrate among 43,960 men who participated in the Health Professionals Follow-Up Study over an 18-y follow-up period. No association was observed after adjustment for confounding, between quintiles of percentage energy from total, animal, or vegetable protein and risk of any stroke type across the range of intake in this cohort. We did find a modestly stronger positive association for total protein and risk of total and ischemic stroke among men with incident hypercholesterolemia compared with those without hypercholesterolemia.

In the context of the current literature

The association between dietary protein and risk of stroke has been investigated in several Japanese populations (1–3). In a population-based Japanese cohort (68 cases/4775 participants), there was a suggestion of an inverse association between total and animal protein and risk of intraparenchymal hemorrhage; however, the RRs were not statistically significant (1). In the Adult Health Study, a cohort of 3731 Japanese participants, there was an inverse association between total and animal protein and risk of fatal ischemic stroke (n = 60) and no association for vegetable protein (3). However, the RR for animal protein became nonsignificant after adjustment for animal fat intake. In the Hiroshima/Nagasaki Life Span Study the RR for daily consumption of animal products (beef, pork, chicken, milk, eggs, dairy, and fish) compared with nonconsumption was 0.88 (95% CI: 0.77, 1.00) for total stroke death, 0.76 (95% CI: 0.58, 0.99) for fatal intracerebral hemorrhage, and 0.89 (95% CI: 0.73, 1.09) for fatal ischemic stroke (2).

Most of the studies in Japanese populations suggest an inverse relation between dietary protein, especially animal protein, and risk of hemorrhagic stroke. We did not observe this inverse relation when we examined the association between protein and intraparenchymal hemorrhage in our cohort. A comparison of our results with those of these other studies is difficult because our study examined the substitution of dietary protein for carbohydrate, whereas the above-mentioned studies examined an increase in the absolute amount of protein in the diet. Only one of the above-mentioned studies presented RRs that were adjusted for dietary fat, which is a factor that has been shown to be inversely associated with stroke risk (1, 3). After adjustment for dietary fat in the Adult Health Study, there was no longer a significant association between animal protein and risk of hemorrhagic stroke (3). It is important to note that the absolute amount of animal protein consumed in the Japanese studies was far lower than the amount of animal protein consumed in the Health Professionals Follow-Up Study cohort.

The association between dietary protein and risk of stroke has also been examined in a US population of female nurses (13). After 14 y of follow-up in 85,764 women, the RR for intraparenchymal hemorrhage (n = 74) when extreme quintiles of intake were compared was 0.47 (95% CI: 0.20, 1.11) for animal protein and 0.81 (95% CI: 0.40, 1.63) for vegetable protein (13). After adjustment for dietary fats and vegetable protein, the RR for animal protein was 0.32 (95% CI: 0.10, 1.00). RRs for vegetable protein after adjustment for fat intake were not presented.

Biological mechanisms

Dietary protein intake may decrease the risk of stroke through its favorable effects on blood pressure, which is an important stroke risk factor (4). Several observational studies have shown an inverse relation between dietary protein intake, especially vegetable protein, and blood pressure (14, 15). A cross-sectional study in a Chinese population found an inverse association between animal protein and blood pressure (16). However, fish was the primary source of animal protein in this study population,

whereas red meat and poultry tend to be more commonly consumed in Western populations. Additionally, the mean percentage energy from protein in the Chinese population was lower than the median percentage energy in our bottom quintile. Randomized clinical trials have also shown an inverse association between high soy protein intake and blood pressure (17–20). However, the subjects received ≈25% of energy from soy protein, which is much greater than the vegetable protein intake in our cohort (median = 6.5% of energy in the fifth quintile).

There are several biological explanations as to why vegetable protein may be protective against stroke, especially ischemic stroke. It has been shown that compared with animal protein, vegetable protein is generally higher in the content of the nonessential amino acids arginine, glycine, alanine, and serine and lower in the content of the essential amino acids methionine, lysine, and tryptophan (21). The intake of essential amino acids results in increased insulin release to stimulate protein synthesis and storage, whereas intake of nonessential amino acids results in gluconeogenesis and therefore decreased insulin levels (21). Higher levels of fasting insulin and type II diabetes have been associated with an increased risk of stroke (22, 23). Furthermore, a higher intake of the amino acid arginine may increase concentrations of the endogenous vasodilator nitric oxide and may decrease blood pressure (17). Thus, the modest inverse association between vegetable protein and risk of ischemic stroke in our study may be biologically plausible and of clinical importance if intakes were substantially higher.

Limitations

The study population for this analysis consisted of US white male health professionals; thus, the results of this study may not be applicable to other racial or ethnic groups. However, despite this limitation the results of this study do extend the current literature because most of the prior work on this topic in men has been conducted in Japanese populations. Another limitation of this study is that death certificates were used to classify stroke subtype for fatal events; thus, misclassification could have occurred. However, it has been suggested that given the increase in the use of computerized tomography to diagnose stroke, death certificates may classify stroke subtypes with reasonable accuracy for observational studies (24). The number of hemorrhagic strokes in our study was small; thus, there may not have been sufficient power to detect a modest association. The intermediate conditions (hypertension, hypercholesterolemia, and diabetes) considered in this study were self-reported; thus, misclassification could have occurred. However, the accuracy of self-reported cardiovascular disease risk factors has been validated in an all-female cohort that used a similar questionnaire (25). Another limitation of our study was the use of an FFQ, which is an inevitably imperfect measure of diet. In a prior validation study the correlation between protein intake estimated from the FFQ and protein intake estimated from diet records was relatively low (0.44) (8). However, the range in protein intake was narrow, which likely contributed to the low correlation. Additionally, a study that compared dietary protein measured by an FFQ and by a urinary nitrogen biomarker showed that underreporting of protein intake was minimal after adjustment for total energy intake (26). All protein measures used in this analysis were adjusted for total energy intake.

Conclusions

We found no significant association between quintiles of percentage of energy from total, animal, or vegetable dietary protein and risk of stroke at the protein amounts consumed in the cohort of US male health professionals. Further examination of the effect of individual amino acids is warranted, as is the exploration of the effects of protein in populations with broader ranges of intake.

The authors' responsibilities were as follows—SRP, MJS, WCW, and EBR: study design; MJS, WCW, and EBR: data collection; SRP and DS: data analysis; SRP: writing of manuscript; and DS, MJS, WCW, and EBR: critical revision of manuscript. None of the authors had a conflict of interest.

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