Dietary Cysteine and Other Amino Acids and Stroke Incidence in Women

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Background and Purpose—Cysteine could potentially lower the risk of stroke through antihypertensive and antioxidant effects. Our aim was to evaluate the hypothesis that cysteine intake is inversely associated with stroke incidence.

Methods—We used data from the Swedish Mammography Cohort, a population-based prospective cohort of 34 250 women who were free of cardiovascular disease and cancer and had completed a food-frequency questionnaire about diet and other risk factors for stroke in the autumn of 1997. Stroke cases were identified by linkage of the study population with the Swedish Inpatient Register and the Swedish Cause of Death Register. Relative risks (RR) with 95% confidence intervals, adjusted for potential confounders, were estimated by using Cox proportional hazards regression model.

Results—We ascertained 1751 incident cases of stroke during 10.4 years of follow-up. Dietary cysteine intake (mean, 635 mg/d) was inversely associated with stroke risk. The multivariable RR of total stroke comparing the highest with the lowest quintile of cysteine intake was 0.79 (95% confidence interval, 0.65–0.97; *P* for trend=0.04). The corresponding RR was 0.82 (95% confidence interval, 0.65–1.03; *P* for trend=0.12) for cerebral infarction and 0.54 (95% confidence interval, 0.29–1.03; *P* for trend=0.08) for intracerebral hemorrhage. Dietary intake of other amino acids showed no independent (after adjustment for cysteine intake) association with stroke risk.

Conclusions—These findings suggest that dietary cysteine intake may be inversely associated with risk of stroke.

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Key Words: 14-3-3 proteins ■ amino acids ■ blood pressure ■ hypertension ■ prospective studies ■ stroke

Evidence from animal studies, randomized trials, and observational studies indicates that dietary protein intake is inversely associated with blood pressure^{1–3} and the risk of stroke.^{4,5} A recent meta-analysis of 7 prospective studies found that intake of total and animal protein, but not vegetable protein, was significantly inversely associated with stroke risk.⁴ Proteins in the diet are composed of several amino acids, each with different chemical properties. Cysteine is a nonessential sulfur-containing amino acid that may, at least in part, be responsible for the potential blood pressure and stroke-reducing effects of a high-protein diet. It is more abundant in animal and cereal proteins than in legume proteins.⁶ Foods rich in cysteine include poultry, egg, beef, and whole grains.⁶ Cysteine may exert its antihypertensive effects directly or via its storage form, glutathione, by reducing oxidative stress and advanced glycation end products, improving insulin sensitivity and glucose metabolism, and modulating nitric oxide levels.⁷ To the best of our knowledge, no study has examined the association between cysteine intake and risk of stroke.

In a previous study, based on data from the Swedish Mammography Cohort (SMC), we observed inverse associations of total and animal protein intakes with stroke incidence.⁸

In this study, we evaluated the a priori hypothesis that cysteine intake is inversely associated with risk of stroke, and that cysteine may have contributed to our previous observed finding for dietary protein. We also provide results for other amino acids, most of which are highly correlated with protein intake, in relation to stroke risk.

Methods

Study Population

This study was based on data from the SMC, which is a population-based prospective cohort study of Swedish women.⁹ In the autumn of 1997, 56 030 participants of the SMC obtained a comprehensive questionnaire that included ≈350 items on diet, other lifestyle factors, etc; 39 227 women (70%) returned a completed questionnaire. We excluded women with an erroneous or a missing personal identification number (*n*=243), women who died before start of follow-up (January 1, 1998; *n*=26), women with a diagnosis of cardiovascular disease (*n*=2492) or cancer (*n*=1811) before start of follow-up, and women with an implausibly high or low energy intake (ie, 3 SDs from the log_e-transformed mean energy intake; *n*=405). After exclusions, 34 250 women aged 49–83 years remained for analysis. The study was approved by the Regional Ethical Review Board at the Karolinska Institutet in Stockholm.

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Baseline Data Collection

The questionnaire inquired about education level, body weight and height, smoking status and history, physical activity, aspirin use, history of diabetes mellitus and hypertension, family history of myocardial infarction before 60 years of age, alcohol consumption, and diet. Participants were classified as having diabetes mellitus if they had a diabetes mellitus diagnosis recorded in the Swedish National Inpatient Register or the Swedish National Diabetes Register, or reported in the questionnaire that they had diabetes mellitus. History of hypertension was based on self-report only. We computed body mass index (BMI) as the weight in kilograms divided by the square of height in meters. Pack-years of smoking history were calculated as the number of packs of cigarettes smoked daily multiplied by the number of years of smoking. The physical activity questions included level of activity at work, home/housework, walking/bicycling, and exercise. There were also questions about inactivity, including watching TV/reading, sleeping, and sitting/lying down. The reported time spent on every activity was multiplied by the activity's typical energy expenditure requirement expressed in metabolic equivalents (MET), and added up to create a MET-hours per day (24 hours) score.¹⁰

Dietary Assessment

Diet was assessed with a 96-item food-frequency questionnaire on which participants reported how often, on average, they had consumed various foods/food items during the past year. There were 8 predefined frequency categories ranging from never to $\geq 3\times$ times per day. Intake of amino acids and other nutrients was calculated by multiplying the frequency of intake of each food by the nutrient content of age-specific (<53, 53–65, and ≥ 66 years) portion sizes, using composition values from the Swedish Food Administration Database. All amino acids and nutrients (except alcohol) were adjusted for total energy intake by using the residual method.¹¹ The food-frequency questionnaire has been validated, and the Spearman correlation coefficients between estimates from the questionnaire and the average of fourteen 24-hour recall interviews was 0.44 for dietary protein.¹²

Case Ascertainment and Follow-Up

By linkage of the study population to the Swedish Inpatient Register and the Swedish Cause of Death Register, we obtained information on stroke events and stroke deaths that occurred in the study cohort. Diagnoses in the Swedish Inpatient Register are coded according to the Swedish *International Classification of Disease* system (adapted from the World Health Organization *International Classification of Disease* classification system). We classified the strokes as cerebral infarctions (*International Classification of Disease*-Tenth Revision code I63), intracerebral hemorrhages (I61), subarachnoid hemorrhages (I60), and unspecified strokes (I64). We received information on dates of death from the Swedish Death Registry.

Statistical Methods

Follow-up time for each participant was computed from January 1, 1998 to the date of first stroke event, death, or end of follow-up (December 31, 2008), whichever occurred first. Cox proportional hazard regression models with age as the time scale was used to estimate relative risks (RRs) and 95% confidence intervals (CIs) of stroke by quintiles of amino acid intake. Entry time was defined as a woman's age in months at start of follow-up, and exit time was defined as a woman's age in months at censoring. The proportional hazards assumption was tested and was found to be met. In a first multivariable model, we adjusted for a combination of smoking status and pack-years of smoking (never [reference]; past <20, 20–39, or ≥ 40 pack-years; or current <20, 20–39, or ≥ 40 pack-years), education (less than high school, high school, or university), BMI (<20, 20–24.9 [reference], 25–29.9, or ≥ 30 kg/m²), total physical activity (quartiles), history of hypertension (yes or no), history of diabetes mellitus (yes or no), aspirin use (yes or no), family history of myocardial infarction before 60 years of age (yes or no), total energy intake (continuous in kcal/d), and quintiles of alcohol and dietary cholesterol intake. In a second multivariable model, we further controlled for quintiles of total protein intake. Adjustment for dietary fiber and nutrients, including dietary fat, saturated fat, carbohydrates, vitamin C, dietary folate, magnesium, potassium, and calcium did not change the results materially. Therefore, those nutrients were not included in the final model. Likewise, we did not include history of hypercholesterolemia

Table 1. Age-Standardized Baseline Characteristics* of 34 250 Women in the Swedish Mammography Cohort by Quintiles of Energy-Adjusted Cysteine Intake

	Cysteine Intake, mg/d				
	<559 (524)†	559–603 (583)	604–645 (624)	646–702 (671)	≥ 703 (755)
No. of women	6849	6852	6863	6831	6855
Age, y	60.7	60.5	61.0	61.5	62.5
Education, university, %	18.9	18.6	19.9	20.1	20.8
Current smoker, %	27.4	27.5	22.4	21.2	21.4
Body mass index, kg/m ²	24.4	24.8	24.9	25.2	25.7
Total physical activity, MET-h/d	42.3	42.7	42.7	42.7	42.6
History of hypertension, %	16.6	18.0	19.2	20.8	23.4
Diabetes mellitus, %	1.57	2.09	2.41	3.93	7.59
Aspirin, any use, %	49.5	48.8	49.6	50.0	48.8
Family history of myocardial infarction, %	15.2	16.0	16.0	17.0	18.6
Alcohol consumption, g/d	8.49	6.84	6.44	5.86	5.61
Total energy intake, kcal/d	1770	1750	1720	1710	1770
Total protein intake, g/d	59.8	66.5	69.9	73.5	83.4
Dietary cholesterol intake, mg/d	216	229	233	237	253

MET indicates metabolic equivalent of energy expenditure (kcal/[kg h]).

*Values are means if not otherwise indicated.

†Median values in parenthesis.

and hormone replacement therapy use in the final model because adjustment for those variables did not alter the results.

We performed tests for trend by assigning the median value of cysteine intake to each quintile and modeled this variable as a continuous variable. Tests for interaction between cysteine intake and history of hypertension (yes or no) or age (median split; <60 versus ≥60 years of age) in relation to stroke risk were conducted by using the likelihood ratio test. All analyses were performed using SAS, version 9.3 (SAS Institute Inc, Cary, NC). All statistical tests were 2-tailed; $P < 0.05$ was considered statistically significant.

Results

Baseline characteristics of study participants according to quintiles of dietary cysteine intake are shown in Table 1. Mean ($\pm$ SD) intake of cysteine was 635 ($\pm$ 101) mg/d. Compared with women in the lowest quintile of cysteine intake, those in the highest quintile tended to be older, had a higher BMI, and were less likely to be current smoker but more likely to have a history of hypertension, a history of diabetes mellitus, and a family history of myocardial infarction. In addition, they consumed less alcohol and had higher intakes of protein and cholesterol. The Spearman correlation coefficient between intake of individual amino acids ($n=18$) and total protein was >0.90 for all amino acids except cysteine ($r=0.71$), glycine ($r=0.77$), proline ($r=0.87$), and arginine ($r=0.88$). Cysteine intake was

more correlated with intake of animal protein ($r=0.50$) than vegetable protein ($r=0.32$).

We ascertained 1751 incident cases of first stroke during a mean follow-up of 10.4 years (355 396 person-years). The stroke cases included 1311 cerebral infarctions, 169 intracerebral hemorrhages, 95 subarachnoid hemorrhages, and 176 unspecified strokes. The association between cysteine intake and risk of total stroke and stroke types are presented in Table 2. Cysteine intake was statistically significantly inversely associated with risk of total stroke after adjustment for other stroke risk factors and after additional adjustment for total protein intake. A high cysteine intake was associated with a reduced risk of cerebral infarction, intracerebral hemorrhage, and total hemorrhagic stroke, but none of the associations were statistically significant after further adjustment for protein intake (model 2).

The Spearman correlation coefficients between intake of cysteine and other amino acids ranged from 0.51 for proline to 0.84 for arginine (Table 3). Intake of most amino acids was statistically significantly inversely associated with total stroke risk after adjustment for other risk factors (multivariable model 1), but none of these associations persisted after additional adjustment for dietary cysteine (Table 3). The inverse association between cysteine intake and stroke remained (multivariable RR for highest versus lowest quintile ranged

Table 2. Relative Risks (95% Confidence Intervals) of Total Stroke and Stroke Types by Quintiles of Energy-Adjusted Cysteine Intake in the Swedish Mammography Cohort ($n=34\,250$), 1998 to 2008

	Quintile of Cysteine Intake, mg/d					<i>P</i> -Trend
	<559	559–603	604–645	646–702	≥703	
Total stroke						
Person-years	70 698	71 328	71 729	70 825	70 816	
No. of cases	363	334	301	375	378	
Age-adjusted model	1.00	0.95 (0.82–1.10)	0.80 (0.69–0.94)	0.97 (0.84–1.12)	0.88 (0.76–1.02)	0.16
Multivariable model 1*	1.00	0.94 (0.80–1.09)	0.77 (0.66–0.90)	0.91 (0.79–1.06)	0.77 (0.66–0.89)	0.0009
Multivariable model 2†	1.00	0.96 (0.82–1.13)	0.81 (0.68–0.96)	0.95 (0.80–1.14)	0.79 (0.65–0.97)	0.04
Cerebral infarction						
No. of cases	262	254	223	288	284	
Age-adjusted model	1.00	1.00 (0.85–1.19)	0.82 (0.69–0.98)	1.02 (0.87–1.21)	0.91 (0.77–1.07)	0.36
Multivariable model 1*	1.00	0.98 (0.82–1.16)	0.78 (0.65–0.93)	0.94 (0.79–1.12)	0.76 (0.64–0.90)	0.003
Multivariable model 2†	1.00	1.01 (0.84–1.21)	0.82 (0.67–1.00)	0.99 (0.80–1.20)	0.82 (0.65–1.03)	0.12
Intracerebral hemorrhage						
No. of cases	38	32	32	34	33	
Age-adjusted model	1.00	0.86 (0.54–1.38)	0.81 (0.51–1.30)	0.84 (0.53–1.34)	0.75 (0.47–1.20)	0.26
Multivariable model 1*	1.00	0.88 (0.55–1.42)	0.83 (0.52–1.34)	0.88 (0.55–1.41)	0.76 (0.47–1.23)	0.30
Multivariable model 2†	1.00	0.81 (0.49–1.34)	0.73 (0.43–1.25)	0.75 (0.43–1.30)	0.54 (0.29–1.03)	0.08
Total hemorrhagic stroke‡						
No. of cases	61	52	47	53	51	
Age-adjusted model	1.00	0.86 (0.59–1.24)	0.75 (0.51–1.09)	0.83 (0.57–1.20)	0.75 (0.52–1.09)	0.16
Multivariable model 1*	1.00	0.88 (0.60–1.27)	0.76 (0.52–1.12)	0.87 (0.60–1.27)	0.77 (0.52–1.13)	0.22
Multivariable model 2†	1.00	0.82 (0.56–1.22)	0.69 (0.45–1.06)	0.76 (0.49–1.18)	0.60 (0.36–1.01)	0.07

*Model 1 was adjusted for age, smoking status and pack-years of smoking, education, body mass index, total physical activity, history of hypertension, history of diabetes mellitus, aspirin use, family history of myocardial infarction, and intakes of total energy, alcohol, and dietary cholesterol.

†Model 2 was adjusted for the same variables as model 1 and further for quintiles of total protein intake.

‡Intracerebral hemorrhage ($n=169$ cases) and subarachnoid hemorrhage ($n=95$ cases) combined.

Table 3. Multivariable Relative Risks (95% Confidence Intervals) of Total Stroke by Energy-Adjusted Amino Acid Intake in the Swedish Mammography Cohort (n=34 250), 1998 to 2008

Amino Acid	Mean Intake, mg/d	SD	Correlation With Cysteine Intake	Highest vs Lowest Quintile	
				Model 1*	Model 2†
Glutamic acid	12 779	2121	0.70	0.83 (0.72–0.97)	0.96 (0.79–1.16)
Aspartic acid	5661	921	0.74	0.79 (0.68–0.92)	0.89 (0.73–1.09)
Leucine	5486	1067	0.65	0.84 (0.72–0.98)	0.96 (0.80–1.16)
Proline	5387	1130	0.51	0.89 (0.76–1.03)	0.99 (0.84–1.17)
Lysine	4695	966	0.63	0.89 (0.76–1.04)	1.03 (0.86–1.25)
Valine	4263	805	0.62	0.84 (0.72–0.98)	0.96 (0.80–1.15)
Serine	3346	596	0.70	0.84 (0.72–0.98)	0.98 (0.81–1.20)
Isoleucine	3282	632	0.67	0.86 (0.74–1.00)	1.00 (0.82–1.21)
Arginine	3189	559	0.84	0.86 (0.74–1.00)	1.13 (0.88–1.45)
Phenylalanine	3161	561	0.70	0.85 (0.73–0.99)	1.01 (0.82–1.23)
Alanine	2992	560	0.82	0.83 (0.71–0.97)	1.04 (0.82–1.33)
Threonine	2575	482	0.74	0.88 (0.75–1.02)	1.07 (0.87–1.31)
Tyrosine	2528	576	0.52	0.88 (0.75–1.03)	0.99 (0.83–1.18)
Glycine	2499	439	0.82	0.83 (0.71–0.96)	0.99 (0.78–1.25)
Histidine	1794	331	0.67	0.89 (0.76–1.04)	1.08 (0.89–1.32)
Methionine	1597	336	0.61	0.85 (0.73–0.99)	0.97 (0.80–1.17)
Tryptophan	808	137	0.66	0.83 (0.71–0.96)	0.95 (0.78–1.15)

*Model 1 was adjusted for age, smoking status and pack-years of smoking, education, body mass index, total physical activity, history of hypertension, history of diabetes mellitus, aspirin use, family history of myocardial infarction, and intakes of total energy, alcohol, and dietary cholesterol.

†Model 2 was adjusted for the same variables as model 1 and further for quintiles of cysteine intake.

from 0.70 to 0.83) in multivariable models adjusted for any of the other 17 amino acids.

We next examined whether dietary cysteine may account for our previously observed inverse relation between total protein intake and risk of stroke.⁸ The multivariable (model 1) RR of total stroke for the highest versus lowest quintile of protein intake was 0.82 (95% CI, 0.70–0.95). This association did not remain after adding cysteine intake (in quintiles) to the model (corresponding RR=0.95; 95% CI, 0.77–1.16).

Finally, we examined whether the association between cysteine intake and risk of stroke was modified by history of hypertension. The multivariable (model 2) RRs of total stroke for the highest compared with the lowest quintile of cysteine intake were 0.75 (95% CI, 0.59–0.96) in women without hypertension at baseline and 0.88 (95% CI, 0.62–1.25) in those with hypertension (*P* for interaction=0.07). We found no interaction between cysteine intake and age in relation to stroke risk (*P* for interaction=0.70).

Discussion

Findings from this prospective study in women suggest that a high cysteine intake may lower the risk of stroke. Women in the highest quintile of cysteine intake had a 21% lower risk of stroke compared with those in the lowest quintile. Our results further suggest that dietary cysteine may account, at least in part, for our previous observed inverse associations of total protein and animal protein intake with risk of stroke.⁸ None of the other 17 amino acids was independently associated with stroke risk.

Rich food sources of cysteine are poultry, egg, beef, and whole grains.⁶ As consumption of red meat and processed meat is positively associated with risk of stroke,¹³ dietary cysteine should preferably be obtained from nonmeat sources.

A reduction in blood pressure and thus, in the risk of stroke by an increased cysteine intake is biologically plausible. It has been proposed that cysteine and its storage form, glutathione, could exert antihypertensive effects by modulating the levels of nitric oxide (NO) and other vasoactive molecules, as well as by reducing oxidative stress, insulin resistance, and advanced glycation products.⁷ Although cysteine is one of the least abundant amino acids, it often occurs in the functional sites of proteins.¹⁴ For example, cysteine is a component of endothelial nitric oxide synthase,¹⁵ which catalyzes the conversion of L-arginine to NO. NO is involved in the regulation of vascular tone.¹⁶ Several studies have shown that dietary supplementation with the cysteine derivative *N*-acetylcysteine (NAC) can prevent or attenuate increased blood pressure in animal models of hypertension.^{7,17} In addition, using NAC as an adjunct to other hypertensive therapies leads to a decrease in blood pressure in humans.^{7,18} The availability of cysteine is a major determinant of the biosynthesis of glutathione, which is an important antioxidant.¹⁹ Hence, cysteine may, in part, lower stroke risk by a reduction in oxidative stress.

In this study, the inverse association between cysteine intake and stroke seemed to be stronger in women without hypertension at baseline. The weaker association in those with hypertension might be due to the antihypertensive effects of cysteine that are diminished by the use of antihypertensive drugs.

To our best knowledge, there are no previous epidemiological studies on dietary amino acids in relation to stroke risk, but the Rotterdam Study²⁰ and the International Collaborative Study of Macronutrients, Micronutrients, and Blood Pressure (INTERMAP)^{21,22} examined the association between intake of certain amino acids (as a percentage of total protein intake) and blood pressure. The Rotterdam Study suggested no major role for cysteine, glutamic acid, arginine, lysine or tyrosine intake in determining blood pressure in a cross-sectional analysis.²⁰ There was also no association between intake of the 5 amino acids and risk of developing hypertension during 6 years of follow-up.²⁰ Mean cysteine intake was much higher in the Rotterdam Study (~600 mg cysteine per 1000 kcal) than in our study (~360 mg cysteine per 1000 kcal). Increased cysteine intake may only have an effect in those with low intakes. In cross-sectional analyses of the INTERMAP, glutamic acid intake was inversely²¹ and glycine intake positively²² associated with blood pressure, whereas no association was observed for phenylalanine, proline, cysteine, or serine intake.²¹ The positive association of dietary glycine with blood pressure was stronger in Western participants, where meat was the main source of glycine, than in East Asian participants, where the main sources were grains/flour, rice/noodles, and fish/shellfish.²²

Major strengths of this study are the prospective and population-based design and the large number of incident stroke cases. Limitations include the use of a self-administered questionnaire to assess diet and other stroke risk factors. Given the prospective design, any measurement error in the assessment of amino acid intake would most probably be unrelated to the outcome (stroke). Nondifferential misclassification of cysteine intake may have resulted in attenuated risk estimates. Another limitation is that information on stroke diagnoses was obtained from the Swedish Inpatient Register and the Cause of Death Register, which are not 100% complete.²³ Thus, there may be some misclassification of stroke cases. Finally, because of the observational design of the study, we cannot rule out that the observed findings are explained by confounding because of measurement error in the variables that we adjusted for or to unmeasured confounders. For example, we did not have information on family history of stroke. Furthermore, we cannot exclude the possibility that cysteine intake represents a certain dietary pattern.

In conclusion, we observed an inverse association between dietary cysteine intake and risk of stroke. Our findings further suggest that cysteine, at least partly, may account for the reported inverse relation between animal protein intake and stroke risk.⁴ These results warrant confirmation by other prospective studies and randomized trials.

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Disclosures

None.

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