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Dietary isoflavones, urinary isoflavonoids, and risk of ischemic stroke in women[1–3]

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ABSTRACT

Background: Hormone therapy has been shown to increase risk of ischemic stroke in women. Plant-derived estrogens, particularly soy isoflavones, are known to have some estrogenic effects and have been marketed as natural alternatives to hormone therapy. Concerns have been raised about whether high isoflavone exposure may be related to ischemic stroke risk as well.

Objective: We examined the dietary intake of isoflavones and the urinary excretion of isoflavonoids in relation to risk of ischemic stroke in women.

Design: A prospective cohort study was conducted in 66,832 Chinese women (aged 40–70 y) who had no cardiovascular disease or cancer at baseline. Usual dietary intakes were assessed via in-person interviews with the use of a validated food-frequency questionnaire. Incident strokes were ascertained during follow-up home visits and confirmed by medical records. We also conducted a nested case-control study in postmenopausal women who had never used hormone therapy, including 1422 incident ischemic stroke cases and 1422 controls individually matched by age, date and time of urine sample collection, time since last meal, and use of antibiotics. Urinary isoflavonoids were measured with the use of high-performance liquid chromatography coupled with mass spectrometry.

Results: During a mean follow-up of 10 y, 3110 incident ischemic strokes were verified. Dietary isoflavone intake was associated with increased risk of ischemic stroke; multivariable-adjusted HRs from lowest to highest quintiles were 1.00, 1.05, 1.10, 1.11, and 1.24, respectively (95% CI: 1.08, 1.42; *P*-trend = 0.002). In the case-control study, a similar positive association was observed for dietary isoflavones, but no significant associations were shown for the urinary isoflavonoid concentration [OR: 1.01 (95% CI: 0.77, 1.32) for comparison of extreme quintiles].

Conclusions: A habitually high intake of soy isoflavones may be associated with a modest but significant increase in risk of ischemic stroke in women. However, no association was shown for the urinary excretion of isoflavonoids. *Am J Clin Nutr* 2015;102:680–6.

Keywords: ischemic stroke, phytoestrogen, prospective cohort study, soy isoflavone, women

INTRODUCTION

Stroke is a leading cause of death and adult disability worldwide (1), especially in women, who face higher lifetime risk and a poorer prognosis than do men (2). Substantial evidence has suggested that exposure to exogenous estrogens and estrogenic compounds, such as selective estrogen receptor modulators (SERMs),[8] may increase stroke risk in women (3–6). It is unclear whether a high exposure to plant-based estrogens (phytoestrogens) also increases stroke risk.

Isoflavones, which are mostly derived from soy foods, are a major class of phytoestrogens that have attracted considerable attention as natural alternatives to hormone therapy (7). Soy isoflavones can bind to estrogen receptors and exert estrogen agonist or antagonist properties similar to those of SERMs (8, 9). Soy isoflavones may also have an antioxidant activity and other nonhormonal effects. Like exogenous estrogens, isoflavones have been shown to lower total cholesterol and LDL cholesterol, reduce LDL-cholesterol oxidation, and improve vascular function (10, 11). However, data are lacking on the potential effects of isoflavones on the coagulation system, and it is unclear whether isoflavones share the prothrombotic effects of exogenous estrogens (8, 9, 12–14).

To date, few prospective cohort studies have examined habitual soy isoflavone intake in relation to the incidence or mortality of cardiovascular disease, including stroke, in women. Overall, no significant associations have been shown in US or European populations, where soy consumption is generally low (15–17). Limited studies in Asian women, who typically

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[2] The contents of this article are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

[3] Supplemental Tables 1 and 2 are available from the "Supplemental data" link in the online posting of the article and from the same link in the online table of contents at http://ajcn.nutrition.org.

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[8] Abbreviations used: FFQ, food-frequency questionnaire; SERM, selective estrogen receptor modulator; SWHS, Shanghai Women's Health Study; WHR, waist-to-hip ratio.

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consume more soy foods than Western women do, have shown inconsistent results; one study suggested an inverse association between isoflavone intake and cerebral infarction in Japanese women (18), whereas 2 other studies in Japanese and Singapore Chinese women reported no association of isoflavone intake with stroke mortality (19, 20).

We examined the association between habitual soy isoflavone intake and risk of incident ischemic stroke in the SWHS (Shanghai Women's Health Study), which is a large, prospective cohort study of middle-aged and older Chinese women. We also conducted, within this cohort, a nested case-control study of ischemic stroke in association with the urinary excretion of isoflavonoids, which is a biomarker related to dietary intakes and other individual characteristics, particularly commensal gut microbiota.

METHODS

Study population

From 28 December 1996 through 23 May 2000, the SWHS recruited 74,941 women aged 40–70 y from urban communities in Shanghai, China (participation rate: 92.7%) (21). Baseline surveys were conducted at participants' homes by trained interviewers who were retired medical professionals. Structured questionnaires were used to collect information on sociodemographic factors, diet and lifestyle habits, and medical histories. Anthropometric measurements and biospecimen collections were also conducted at baseline. Weight, height, and circumferences of the waist and hips were measured twice following standardized protocols. Eighty-eight percent of participants provided consent for and donated a spot urine sample (n = 65,753), which was stored at −70°C within 6 h of collection until laboratory assays. Participants were followed up through home visits every 2–3 y (follow-up rate: 92.3%) and annual record linkage to the Shanghai Vital Statistic Registry. The institutional review boards of the Shanghai Cancer Institute and Vanderbilt University approved the study, and all participants provided written informed consent.

Dietary assessment

Habitual dietary intakes were assessed with the use of a validated food-frequency questionnaire (FFQ). A total of 77 food items were included, which covered 90% of commonly consumed foods in urban Shanghai at baseline. For each food item, participants were asked how often and how much food they ate, on average, during the past 12 mo. Energy and nutrient intakes per day were calculated on the basis of the amount of food consumed and the nutrient content estimated with the use of Chinese Food Composition Tables (22). The FFQ captured virtually all soy foods that are commonly consumed in the study population, including soy milk, tofu, fried bean curd, bean curd cake, dry soybeans, fresh soybeans, soybean sprouts, and other soy products. Intakes of isoflavones and soy protein were estimated from these food items and the food-composition table. The FFQ was validated against multiple 24-h dietary recalls recorded twice a month consecutively for 12 mo (23). Correlation coefficients were 0.49 for soy foods, 0.59–0.66 for macronutrients, and 0.41–0.66 for other major food groups.

Outcome ascertainment

Possible stroke cases were identified during follow-up home visits. Medical records were sought for women who reported a first-ever diagnosis of stroke; these records were reviewed by physicians who were unaware of the participant exposure status. The diagnosis of stroke was confirmed according to criteria adapted from the US National Survey of Stroke (24), which required evidence of a sudden or rapid onset of neurologic deficits that persisted for ≥24 h or until death and had no apparent nonvascular causes such as trauma, tumor, or infection. Stroke cases were further classified as ischemic stroke, hemorrhagic stroke, or stroke of an undetermined type on the basis of clinical findings, computerized tomography or an MRI scan of the brain, or angiographic findings. Fatal stroke events were confirmed by a review of medical records and death certificates. The follow-up time was calculated from the date of the baseline interview to the date of stroke diagnosis, death, loss to follow-up, or 31 December 2011, whichever occurred first.

The nested case-control study was conducted in postmenopausal women who had never used hormone therapy, including 1500 women with incident ischemic stroke and 1500 women who remained free of stroke. Cases and controls were matched for age (≤2 y), date of urine-sample collection (≤1 mo), time of sample collection (≤1 h), time interval since last meal (≤2 h), and use of antibiotics in the past 7 d (yes or no).

Measurement of urinary isoflavonoids

Urinary isoflavonoids were measured with the use of HPLC coupled with isotope-dilution electrospray ionization–tandem mass spectrometry (model TSQ Ultra; Thermo Fisher Scientific), as described previously (25). Seven isoflavonoids were quantified including 3 parent compounds (daidzein, genistein, and glycitein) and 4 metabolites (dihydrogenistein, dihydrodaidzein, *O*-desmethylangolensin, and equol). Detection limits were 1 nM for all parent compounds and *O*-desmethylangolensin, 0.4 nM for dihydrogenistein and dihydrodaidzein, and 2 nM for equol. Concentrations under the detection limits were assigned random numbers between zero and the corresponding detection limits. Between-day CVs for all analytes ranged from 4% to 18%, and within-day CVs were all <9%. Urinary creatinine (in mg/L) was measured with the use of a kit from Randox Laboratories with a Roche-Cobas MiraPlus chemistry autoanalyzer. To control for between-batch variations, case-control pairs were analyzed in the same assay run with a random and blind arrangement.

Statistical analysis

For the cohort analysis, we excluded 7999 women who had a history of cardiovascular disease or cancer at baseline. We also excluded 110 women whose energy intakes were either <500 or >3500 kcal/d. A total of 66,832 women were included in the analyses.

Dietary intakes were adjusted for total energy with the use of the residual method (26). Baseline characteristics by quintiles of isoflavone intake were compared by ANOVA for continuous variables and the chi-square test for categorical variables. HRs and 95% CIs were estimated with the use of a Cox proportional hazards model with age as the timescale. The Cox model was

stratified by birth cohort (5-y interval) and adjusted for potential confounders, including income (4 categories), education (4 categories), leisure-time physical activity (quartiles of metabolic equivalent task hours per day), cigarette smoking (yes or no), alcohol consumption (yes or no), BMI (in kg/m^2), waist-to-hip ratio (WHR), menopause, use of hormone therapy, history of diabetes, history of hypertension, use of antihypertensive medication, family history of stroke, and intakes of total energy, carbohydrate, and saturated fat. A linear trend was tested by treating the median value of each quintile as a continuous variable. The dose-response relation between isoflavones and ischemic stroke was further examined with the use of a restricted cubic spline regression. Three knots were chosen on the basis of a smaller Akaike Information Criterion (10th, 50th, and 90th percentiles), and the median intake was used as the referent. To evaluate a potential effect modification and robustness of the association, stratified analyses were performed by age, education, menopause, BMI, WHR, and previous hypertension or diabetes. Sensitivity analyses were conducted by omitting the first 2 y of follow-up.

For the nested case-control study, we excluded 40 cases and 41 controls with extreme urinary creatinine concentrations (<10 or >350 mg/dL) and analyzed 1422 case-control pairs. The urinary excretion of isoflavonoids was expressed in nmol/mg creatinine and log transformed before analysis. Baseline characteristics were compared with the use of paired *t* tests. Participants were classified into quintiles according to isoflavone intakes or isoflavonoid excretion concentrations in controls. A conditional logistic regression was used to estimate ORs with adjustment for all confounders included in the cohort analysis except menopause and hormone-therapy use. A 2-sided $P < 0.05$ was considered statistically significant. All tests were conducted with SAS 9.3 software (SAS Institute), and graphics were plotted with the R package ggplot2 (27).

RESULTS

In the SWHS, the median intake of soy isoflavones was 25.0 mg/d with a 10th through 90th percentile range of 9.0–53.6 mg/d. Compared with women who consumed less soy isoflavones, women in the highest quintile of intake were older and had lower income and educational levels but higher BMI, WHR, and physical activity levels (**Table 1**). Women in the highest quintile of intake were also more likely to have a history of diabetes or hypertension and a family history of stroke. Isoflavone intake was associated with a lower intake of carbohydrates but a higher intake of saturated fat.

During a mean follow-up of 10 y, we verified 3110 incident ischemic stroke cases. Higher isoflavone intake was associated with increased risk of ischemic stroke (**Table 2**). After adjustment for potential confounders, HRs were 1.00 (referent), 1.05, 1.10, 1.11, and 1.24 (95% CI: 1.08, 1.42) for lowest to highest quintiles of intake, respectively (*P*-trend = 0.002). Analyses that used restricted cubic splines indicated a linear, dose-response relation between isoflavone intake and ischemic stroke (*P*-linear = 0.01, *P*-nonlinear = 0.18) (**Figure 1**A). Positive associations were shown in all subgroups of women grouped by age, menopausal status, education, BMI, WHR, and disease history (**Figure 2**). Similar but slightly weaker associations were observed for intakes of total soy protein (Table 2), total soy foods (dry weight), and the major soy foods consumed in the SWHS (soy milk and tofu) (**Supplemental Table 1**). Results were basically the same when the first 2 y of follow-up were excluded.

TABLE 1
Baseline characteristics of participants by quintiles of dietary isoflavone intake in the SWHS (n = 66,832)[1]

	Dietary isoflavone intake		
Characteristic	Quintile 1 (low)	Quintile 3	Quintile 5 (high)
Isoflavone intake, mg/d	8.6 ± 3.3[2]	25.0 ± 2.5	59.4 ± 18.7
Age, y	51.0 ± 8.9	51.4 ± 8.6	53.6 ± 9.1
Household income ≥20,000 Yuan/y, %	17.6	18.5	16.3
Education of at least high school, %	40.3	44.9	38.9
Regular cigarette smoking, %	3.1	2.3	3.3
Regular alcohol consumption, %	2.0	2.2	2.9
BMI, kg/m^2	23.7 ± 3.4	23.9 ± 3.3	24.2 ± 3.4
Waist-to-hip ratio	0.808 ± 0.053	0.807 ± 0.052	0.813 ± 0.053
Leisure-time physical activity, metabolic equivalent task hours/d	0.5 ± 1.3	0.6 ± 1.4	0.9 ± 1.8
Postmenopausal, %	41.4	43.9	53.9
Hormone-therapy use, %	1.5	2.0	2.1
History of diabetes, %	1.6	2.2	8.8
History of hypertension, %	16.9	18.8	25.4
Antihypertensive medication use, %	10.2	12.0	17.6
Family history of stroke, %	16.5	17.8	17.5
Total energy intake, kcal/d	1658 ± 405	1712 ± 392	1628 ± 394
Carbohydrate intake, g/d	293 ± 27	280 ± 25	265 ± 27
Saturated fat intake, g/d	7.7 ± 3.2	8.3 ± 2.9	8.7 ± 3.0
Soy protein intake, g/d	2.8 ± 1.2	7.6 ± 1.4	16.5 ± 5.7

[1]ANOVA was used for continuous variables, and the chi-square test was used for categorical variables. *P* values were all <0.05, except for cigarette smoking. SWHS, Shanghai Women's Health Study.

[2]Mean ± SD (all such values).

TABLE 2
Ischemic stroke by quintiles of dietary isoflavone and soy protein intakes in the SWHS (n = 66,832)[1]

	Quintile of intake					
	1 (low)	2	3	4	5 (high)	P-trend
Dietary isoflavones						
Median intake, mg/d	9.0	17.1	24.9	35.0	53.6	—
Cases, n	538	523	567	627	855	—
Age and energy adjusted	1 (referent)	1.01 (0.90, 1.14)[2]	1.05 (0.94, 1.18)	1.09 (0.97, 1.22)	1.26 (1.13, 1.40)	<0.0001
Multivariable model	1 (referent)	1.05 (0.93, 1.18)	1.10 (0.97, 1.24)	1.11 (0.98, 1.26)	1.24 (1.08, 1.42)	0.002
Soy protein						
Median intake, g/d	2.9	5.2	7.4	10.1	15.2	—
Cases, n	535	528	583	634	830	—
Age and energy adjusted	1 (referent)	1.00 (0.89, 1.13)	1.04 (0.93, 1.17)	1.06 (0.94, 1.19)	1.20 (1.07, 1.33)	0.0002
Multivariable model	1 (referent)	1.03 (0.91, 1.16)	1.06 (0.94, 1.20)	1.08 (0.95, 1.22)	1.16 (1.01, 1.33)	0.03

[1]Cox proportional hazards regression was used with age as the time scale stratified by 5-y birth cohorts and in the multivariable model, adjusted for income, education, physical activity, smoking, alcohol consumption, BMI, waist-to-hip ratio, menopausal status, hormone-therapy use, history of diabetes and hypertension, antihypertensive medication use, family history of stroke, and intakes of total energy, carbohydrates, and saturated fat. SWHS, Shanghai Women's Health Study.

[2]HR; 95% CI in parentheses (all such values).

In the nested case-control study, we also showed a positive association between dietary isoflavone intake and risk of ischemic stroke (Figure 1B, **Table 3**). The OR was 1.29 (95% CI: 0.94, 1.76) for highest vs. lowest quintiles (P-trend = 0.02). However, we showed no significant differences in the urinary excretion of isoflavonoids between cases and controls. Across all urinary isoflavonoid concentrations, women had a similar sociodemographic and obesity status, but those with higher urinary isoflavonoid concentrations consumed more isoflavones and had a higher prevalence of diabetes and hypertension (**Supplemental Table 2**). The OR for the comparison of extreme quintiles of urinary isoflavonoid concentrations was 1.01 (95% CI: 0.77, 1.32; P-trend = 0.60) after multivariable adjustments including for a previous history of hypertension and diabetes. Restricted cubic-spline analyses revealed a possible nonlinear association between isoflavonoid excretion and stroke risk (P-linear = 0.64, P-nonlinear = 0.03) (Figure 1C).

DISCUSSION

In this large, prospective cohort study of Chinese women, we observed that a higher dietary soy isoflavone intake was associated with a small but significant increase in risk of ischemic stroke. The association was independent of established stroke risk factors and consistent in various subgroups of women with different baseline risk factors. In a nested case-control study, we showed a similar, positive association of ischemic stroke with dietary soy isoflavone intakes but no association with the urinary excretion of isoflavonoids.

The positive association between dietary isoflavone intake and risk of ischemic stroke shown in our study is in line with reported excess risk of stroke associated with estrogen therapy and SERMs (3–6). Although additional verification is required, our findings provide support for a possible positive association between exposure to estrogenic compounds from dietary sources and risk of ischemic stroke. To date, limited epidemiologic studies have focused on stroke risk associated with phytoestrogens. Results have been mixed and inconclusive. Isoflavone intake was not associated with stroke incidence in Dutch women (15) but showed a positive, although nonsignificant, association with stroke mortality in 2 studies of US women (16, 17). In Asian countries, where soy consumption is traditionally common and relatively high (median isoflavone intake: 15–40 mg/d in Asian populations vs. 0.3–0.4 mg/d in Western populations), increased isoflavone intake was associated with lower stroke incidence in Japanese women (18) but not associated with stroke mortality in women from another Japanese cohort (19) or in women in the Singapore Chinese Health Study (20). The discrepant findings from these studies may be partly explained by differences in the amount of consumption, type of soy products consumed, population characteristics, assessment of isoflavone exposure, and variations in isoflavone metabolism.

To our knowledge, the current study is the first to assess the association of stroke risk with the urinary excretion of isoflavonoids in addition to dietary intake of isoflavones. Urinary isoflavonoids are considered a biomarker of isoflavone exposure and may provide an integrated measure of intake, absorption, metabolism, and elimination (25). One major limitation of our study was the availability of only a single spot urine sample. Because urinary isoflavonoid excretion is a marker of short-term exposure with a half-life of 7–10 h (25), a single measurement may not have adequately reflected usual or long-term average dietary intakes that are likely more relevant to health. The weak correlation between dietary isoflavones and urinary isoflavonoids (r = 0.25) in our study may provide a possible explanation for the inconsistent findings on the associations with dietary isoflavones and urinary isoflavonoids. The urinary excretion of isoflavonoids is determined not only by dietary intake but many others factors (28). As shown in Supplemental Table 2, participants with substantial differences in urinary isoflavonoid concentrations did not differ as strikingly in dietary intakes of isoflavones, which underscored the heterogeneity in isoflavone metabolism in individuals. Growing evidence suggests that commensal gut microbiota play an important role in the metabolism of isoflavones. The health effects of isoflavones may be influenced by the composition of intestinal bacteria and their ability to metabolize isoflavones into various metabolites with different biological activities (29, 30). The inability to account

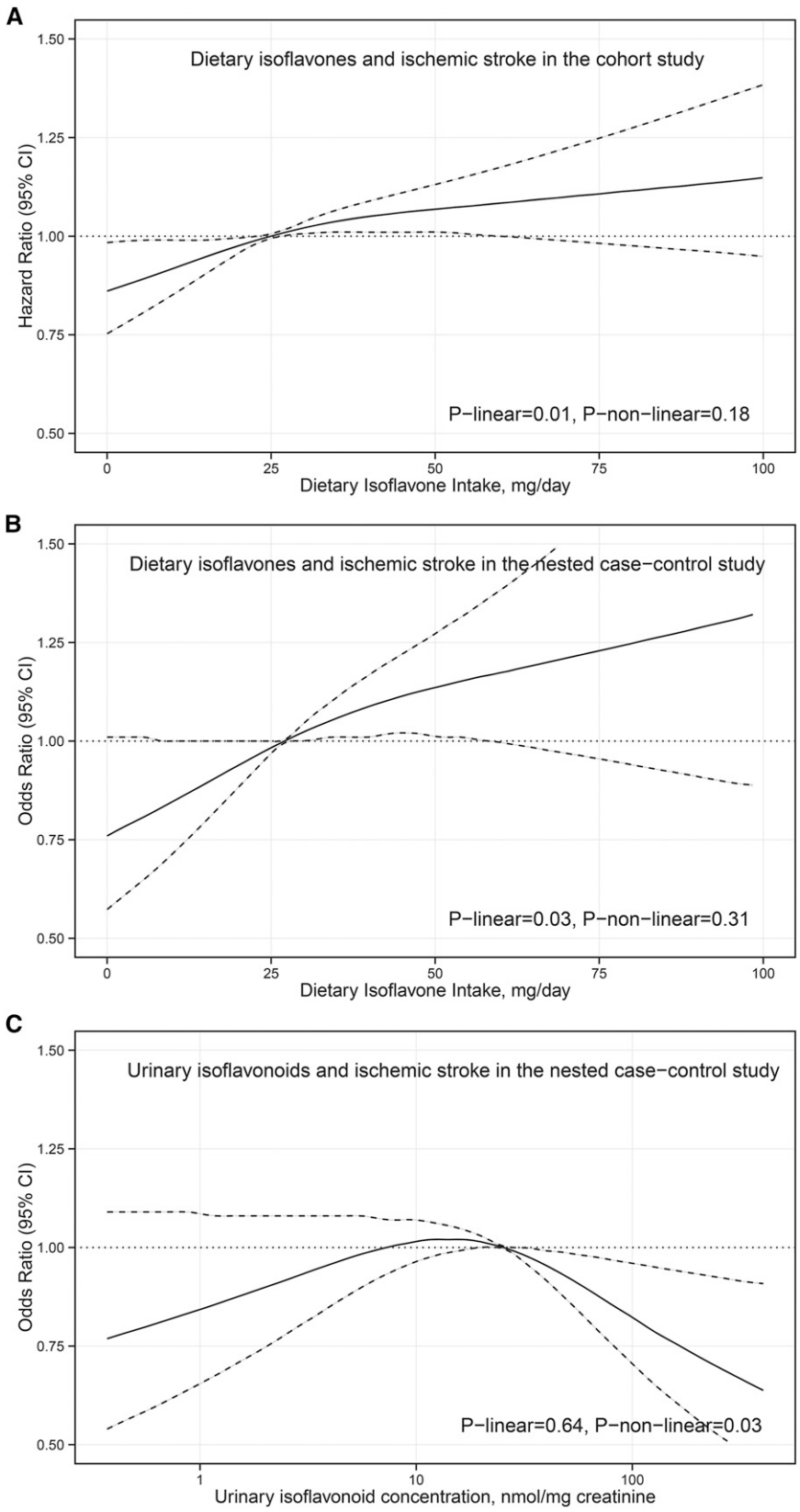

FIGURE 1 Dose-response associations of dietary isoflavone intakes (A and B) and urinary isoflavonoid concentrations (C) with ischemic stroke in the Shanghai Women's Health Study. A restricted cubic spline regression was performed with the use of 3 kn (10th, 50th, and 90th percentages), with the median intake or concentration as the referent, and adjusted for age, income, education, physical activity, smoking, alcohol consumption, BMI, waist-to-hip ratio, menopausal status, hormone-therapy use, history of diabetes and hypertension, antihypertensive medication use, family history of stroke, and intakes of total energy, carbohydrate, and saturated fat. There were 66,832 women in the cohort study and 1422 pairs in the nested case-control study.

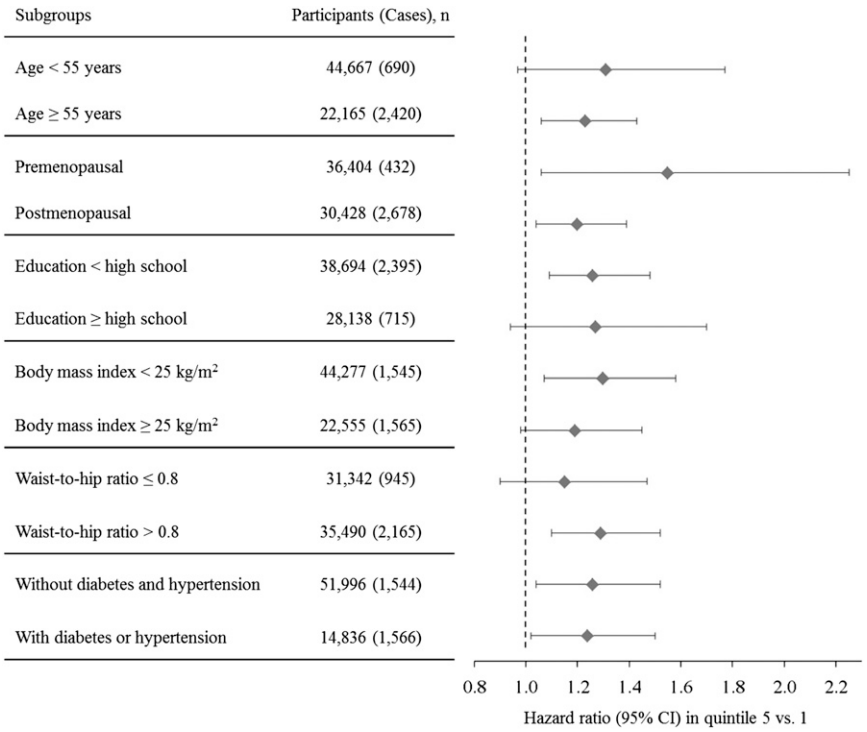

FIGURE 2 HRs (95% CIs) of ischemic stroke by dietary isoflavone intakes in subgroups in the Shanghai Women's Health Study. The proportional hazard model was stratified by 5-y birth cohorts and, wherever applicable, adjusted for income, education, physical activity, smoking, alcohol consumption, BMI, waist-to-hip ratio, menopausal status, hormone-therapy use, history of diabetes and hypertension, antihypertensive medication use, family history of stroke, and intakes of total energy, carbohydrate, and saturated fat. *P*-interaction = 0.97 for age, 0.59 for menopausal status, 0.85 for educational level, 0.63 for BMI, 0.74 for waist-to-hip ratio, and 0.91 for disease status.

for variations in gut microbiota and other factors involved in isoflavone metabolism and excretion may have contributed to the observed null association between urinary isoflavonoids and stroke risk. It is also possible that measurement errors may have reduced the power of our study to detect a moderate association despite the matching of cases and controls by the date and time of urine collection and measurement of urinary isoflavonoids for each case-control pair in the same batch.

In conclusion, in this large, prospective cohort study of Chinese women, we showed that a habitually high intake of soy isoflavones may be associated with increased risk of ischemic stroke. Although excess risk is small, our findings raise concern

TABLE 3
Ischemic stroke by quintiles of dietary isoflavone intake and urinary isoflavonoid concentration in a case-control study nested within the SWHS (n = 1422 pairs)[1]

	Quintile of exposure					
	1 (low)	2	3	4	5 (high)	*P*-trend
Dietary isoflavones						
Median intake, mg/d	9.2	18.0	26.9	39.3	59.8	—
Cases, *n*	281	221	293	296	331	—
Age and energy adjusted	1 (referent)	0.79 (0.62, 1.01)[2]	1.06 (0.83, 1.35)	1.07 (0.84, 1.35)	1.17 (0.93, 1.47)	0.02
Multivariable model[3]	1 (referent)	0.83 (0.63, 1.07)	1.15 (0.88, 1.50)	1.24 (0.94, 1.64)	1.29 (0.94, 1.76)	0.02
Urinary isoflavonoids						
Median excretion, nmol/mg creatinine	2.3	10.3	25.5	53.2	114.4	—
Cases, *n*	248	315	321	257	281	—
Age and energy adjusted	1 (referent)	1.27 (1.01, 1.61)	1.31 (1.02, 1.67)	1.04 (0.82, 1.34)	1.15 (0.90, 1.47)	0.60
Multivariable model[3]	1 (referent)	1.24 (0.97, 1.58)	1.25 (0.97, 1.63)	0.96 (0.74, 1.25)	1.01 (0.77, 1.32)	0.60

[1]Quintile cutoffs were determined by the distribution of energy-adjusted isoflavone intake levels or log-transformed urinary isoflavonoid concentrations in controls. SWHS, Shanghai Women's Health Study.

[2]OR; 95% CI in parentheses (all such values).

[3]Conditional logistic regression model was stratified by case-control pairs and adjusted for age, education, income, physical activity, smoking, alcohol consumption, BMI, waist-to-hip ratio, history of diabetes or hypertension, antihypertensive medication use, family history of stroke, and intakes of total energy, carbohydrates, and saturated fat.

about the safety of high and long-term exposure to isoflavones for stroke prevention. However, we showed no association of risk of ischemic stroke with urinary isoflavone concentrations, which reflect short-term exposure and differences in biological metabolism. The current study is limited by possible measurement errors and residual confounding. Future studies are needed to explore underlying mechanisms and interactions of dietary isoflavones with gut microbiota.

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