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Dietary flavonoid intake and the risk of stroke: a dose-response meta-analysis of prospective cohort studies

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ABSTRACT

Objective: To clarify and quantify the potential association between intake of flavonoids and risk of stroke.

Design: Meta-analysis of prospective cohort studies.

Data source: Studies published before January 2016 identified through electronic searches using PubMed, Embase and the Cochrane Library.

Eligibility criteria for selecting studies: Prospective cohort studies with relative risks and 95% CIs for stroke according to intake of flavonoids (assessed as dietary intake).

Results: The meta-analysis yielded 11 prospective cohort studies involving 356 627 participants and more than 5154 stroke cases. The pooled estimate of the multivariate relative risk of stroke for the highest compared with the lowest dietary flavonoid intake was 0.89 (95% CI 0.82 to 0.97; p=0.006). Dose-response analysis indicated that the summary relative risk of stroke for an increase of 100 mg flavonoids consumed per day was 0.91 (95% CI 0.77 to 1.08) without heterogeneity among studies (I^2=0%). Stratifying by follow-up duration, the relative risk of stroke for flavonoid intake was 0.89 (95% CI 0.81 to 0.99) in studies with more than 10 years of follow-up.

Conclusions: Results from this meta-analysis suggest that higher dietary flavonoid intake may moderately lower the risk of stroke.

INTRODUCTION

Stroke is the second most common cause of death, as well as the fourth leading cause of lost productivity and the second highest cause of disability worldwide.[1][2] Prevention of stroke is thus clearly an important public health priority. In recent decades, concern has mounted regarding premature incidence and mortality associated with stroke, and there is growing interest in altering risk factors and reversing this global epidemic. Among the known risk factors for stroke, dietary factors, especially dietary flavonoid intake, have aroused particular attention. Clinical studies have shown that flavonoid intake reduces cardiovascular disease (CVD)

Strengths and limitations of this study

- This is the largest meta-analysis to date on flavonoid intake and risk of stroke.
- Higher dietary flavonoid intake is associated with a significantly reduced risk of stroke.
- Dose-response analyses indicated a 9% lower risk of stroke per 100 mg/day increment in flavonoids.
- The possibility of residual confounding or confounding by unmeasured factors, which cannot be ruled out in any observational study, must be acknowledged.
- We cannot exclude the possibility of recall bias in the assessments of diet based on food frequency questionnaires.

risk.[3–5] Additionally, experimental studies indicated that flavonoids have both antioxidant and antithrombotic properties.[6][7]

Over the last two decades, many prospective studies have assessed the association between dietary flavonoid intake and risk of stroke.[8–18] Although a recent meta-analysis that combined the results from eight cohort studies of flavonol intake and risk of stroke found a significant association of stroke of 0.86 (95% CI 0.75 to 0.99) for the highest versus lowest category of flavonol intake,[19] the role of flavonoid intake in stroke prevention is still controversial. In addition, flavonoid intake differed substantially between studies, which makes it difficult to interpret the summary estimate based on results from study populations with different flavonoid intakes.[20]

To fill these gaps, we conducted a dose-response meta-analysis of the current evidence for the association between flavonoid exposure, including cohort studies of dietary flavonoids, and risk of stroke.

METHODS
Literature search

The search strategy was conducted according to the Meta-analysis of Observational Studies

in Epidemiology (MOOSE) guidelines.[21] We performed a systematic search of PubMed, Embase and the Cochrane library through February 2015. The following key words were used in our search strategies: 'flavonoids', 'polyphenols', 'phenolics', 'flavonols', 'flavones', 'quercetin', 'kaempferol', 'myricetin', 'isorhamnetin', 'apigenin', 'luteolin', 'proanthocyanidins', 'anthocyanins', 'anthocyanidins', 'flavan-3-ols', 'isoflavones', 'flavanones', 'catechins' and 'stroke', 'cerebrovascular disease', 'cerebrovascular disorders', 'cerebral infarct', 'ischemic stroke', 'intracranial hemorrhage', 'intracranial artery disease', 'cardiovascular disease', 'myocardial ischemia', 'myocardial infarct', 'ischemic heart disease', 'coronary heart disease', and 'longitudinal studies', 'cohort studies', 'prospective studies' and 'follow-up studies'. We restricted the search to human studies. There were no language restrictions. In addition, we reviewed the reference lists of obtained articles and contacted authors to identify additional relevant studies and information. When the same or a similar patient cohort was included in several publications, only the most recent or complete report was selected for analysis. This search strategy was updated on 14 January 2016.

Study selection

Studies were selected for the meta-analysis if they fulfilled the following entry criteria: (1) the study had a community-based or population-based, prospective cohort design; (2) the exposure of interest was intake of dietary flavonoids (including flavonols, flavones, flavanones, flavan-3-ols, anthocyanidins, proanthocyanidins and isoflavones); (3) the outcome of interest was stroke, including all types of stroke (fatal, non-fatal, ischaemic and haemorrhagic); (4) reported quantitative estimates of the multivariate-adjusted relative risk (RR) and 95% CI for stroke incidence or mortality associated with flavonoid intake; and (5) more than 1 year of follow-up. Studies were excluded if: (1) the study had a cross-sectional or case-control design; (2) reported unadjusted or only age- or sex-adjusted RR; and (3) there was less than 1 year of follow-up.

Data abstraction

All data were independently abstracted in duplicate by two investigators (ML and XZ). Discrepancies were resolved by consensus. When necessary, the original authors were contacted for supplementary information. The following data were extracted from each study: the first author's last name, publication year, country where the study was performed, location, number of participants, participants' age, years of follow-up, assessment of dietary data and stroke, number of cases, adjusted covariates and study quality.

Assessment of study quality

The Newcastle-Ottawa Scale (NOS) was used to assess the quality of studies.[22] The quality of cohort studies was evaluated as regards the following three major

components: selection of the study group (0–4 stars), quality of the adjustment for confounding (0–2 stars) and assessment of outcome in the cohorts (0–3 stars). A higher score represents better methodological quality. The top score was nine stars. Studies were graded as high quality if they were awarded nine stars.

Statistical analysis

RRs were used as the common measure of association between flavonoid intake and stroke, and HRs were considered equivalent to RRs. Data analysis used multivariate-adjusted outcome data. We converted these values in every study by using their natural logarithms, and the SEs were calculated from these logarithmic numbers and their corresponding 95% CIs. When data on total stroke were not available, we used data from ischaemic stroke, non-fatal stroke or fatal stroke as an equivalent to total stroke.[23] We combined these estimates using a random-effects model, which takes into account both within-study and between-study variabilities.[24] In the dose-response analysis, the generalised least square for trend estimation method described by Greenland and Longnecker[25] and Orsini et al[26] was used to calculate study-specific slopes (linear trends) and 95% CIs. The method requires the distributions of cases and person-years for exposure categories, and the median/mean of flavonoid intake level for each comparison group. We assigned the midpoint of the upper and lower boundaries of each comparison group to determine mean flavonoid intake level if the median or mean intake was not provided. If the lower or upper boundary for the lowest and highest category, respectively, was not reported, we assumed that the boundary had the same amplitude as the closest category.[20] Additionally, we first created restricted cubic splines with three knots at fixed percentiles (25%, 50%, and 75%) of the distribution.[27] A p value for non-linearity was calculated by testing the null hypothesis that the coefficient of the fractional polynomials component is equal to zero. When data on total flavonoid intake included in this dose-response meta-analysis were not available, we used data from flavonols, flavones, flavanones, flavan-3-ols and isoflavones (in sequential order) as an equivalent to total flavonoid. Heterogeneity among studies was evaluated using the χ^2 test based on Cochran's Q test and the I^2 statistic at $p<0.10$ level of significance,[24] which describes the percentage of variability in the effect estimates that is due to heterogeneity rather than chance.[28] We regard $I^2<40\%$ as 'heterogeneity that might not be important' and $I^2>75\%$ as 'considerable heterogeneity' based on the suggestion of the *Cochrane Handbook for Systemic Review of Interventions*.[29] All available data were utilised in the primary analysis. Subsequent subgroup analyses were conducted according to stroke outcomes (fatal/non-fatal vs ischaemic), follow-up duration (≤10 years vs >10 years), sex (male vs female vs combined), geographical area (USA vs Asia vs Europe), history of stroke (yes vs no) and study quality (high

(score>8) vs low (score ≤8)). To explore possible explanations for heterogeneity and to test the robustness of the association, we conducted meta-regression, sensitivity analyses and the above-mentioned stratified analysis. The possibility of publication bias was evaluated using the Egger rank correlation test at p<0.10 level of significance and visual inspection of a funnel plot.[30][31] In the case of publication bias, the 'non-parametric trim-and-fill' method was used to compute risk estimates corrected for this bias.[32] All statistical analyses were performed in STATA V.12.0 (StataCorp, College Station, Texas, USA). p Values were two-sided and p<0.05 was considered statistically significant.

RESULTS
Literature search
Figure 1 shows the results of literature search and selection. We initially identified 236 citations. After exclusion of duplicate records and studies that did not fulfil our inclusion criteria, 28 studies remained. We evaluated the full texts of these 28 publications and excluded 17 studies for the following reasons: no stroke outcomes (n=8), duplicate reports (n=4) and reviews (n=5). Eleven studies finally met the inclusion criteria and were included in the meta-analysis.[8–18]

Study characteristics
The characteristics of the studies and of their participants are presented in table 1 and supplementary tables 1 and 2. Eleven studies involving 356 627 participants and more than 5154 stroke cases were included in the meta-analysis.[8–18] Of these 11 studies, four conducted in the USA,[11 14 16 17] one in an Asian country (Singapore)[18] and six in European countries (Finland and the Netherlands).[8–10 12 13 15] The number of participants ranged from 755 in the study by Marniemi et al[12] to 98 469

Figure 1 Literature search and study selection.

in the study by McCullough et al.[17] Four studies included both men and women,[10 12 17 18] three studies included only men[8 9 15] and four studies only women.[11 13 14 16] The duration of follow-up ranged from 6.1 to 28 years,[8 10] with a median of 14 years. The dietary assessment of flavonoid intake varied across studies: in most studies, intake of flavonoids was measured using food frequency questionnaires (FFQs) and dietary history interview. In most studies, stroke was assessed by medical records or death certificates based on ICD-8, ICD-9 or ICD-10.[33–35] All studies provided adjusted risk estimates (eg, sex, body mass index, smoking, education), overall quality scores ranged from 7 to 9, and the median score was 8.

Flavonoid intake and stroke risk
The multivariable adjusted RRs of fatal or non-fatal stroke in relation to dietary flavonoid intake from individual studies and the combined RR are presented in figure 2. Only one of the 11 studies showed that flavonoid intake was significantly associated with decreased risk of stroke.[10] Overall, participants with the highest dietary flavonoid intake experienced a significantly decreased risk for stroke compared with the lowest (combined RR 0.89, 95% CI 0.82 to 0.97; figure 2) after adjustment for other risk factors. We saw no heterogeneity among studies (p=0.774, I^2=0%). Three of the 11 studies were eligible for the dose-response analysis of flavonoid intake and risk of stroke.[9 14 16] Using a restricted cubic splines model, dose-response analysis found a statistically non-significant inverse association with risk of stroke per 100 mg/day increment in flavonoid intake (RR 0.91, 95% CI 0.77 to 1.08; I^2=0%, p=0.285; figures 3 and 4). No heterogeneity was observed (p=0.974).

Stratifying analysis
In most subgroups, dietary flavonoid intake was not associated with stroke. Stratifying by sex, the associations between dietary flavonoid intake and risk of stroke were similar between males and females (table 2). The pooled RRs of stroke were 0.86 (95% CI 0.76 to 0.98) in studies conducted in the USA, 0.88 (95% CI 0.77 to 1.00) in studies conducted in Europe, and 0.97 (95% CI 0.81 to 1.16) in studies conducted in Asia (table 2). Stratifying by duration, we observed a protective effect of dietary flavonoid intake in studies with more than 10 years of follow-up. The associations were more evident in some strata of study characteristics (table 2).

Sensitivity analysis and publication bias
We tested the robustness of our results in sensitivity analysis, where a single study included in the meta-analysis was deleted at a time; the results of the meta-analysis remained largely unchanged, indicating that the results of the meta-analysis were stable (data not shown). Visual inspection of the funnel plot identified asymmetry (see online supplementary figure S1). The Egger test demonstrated no statistical evidence of publication bias among studies for stroke risk (p=0.238).
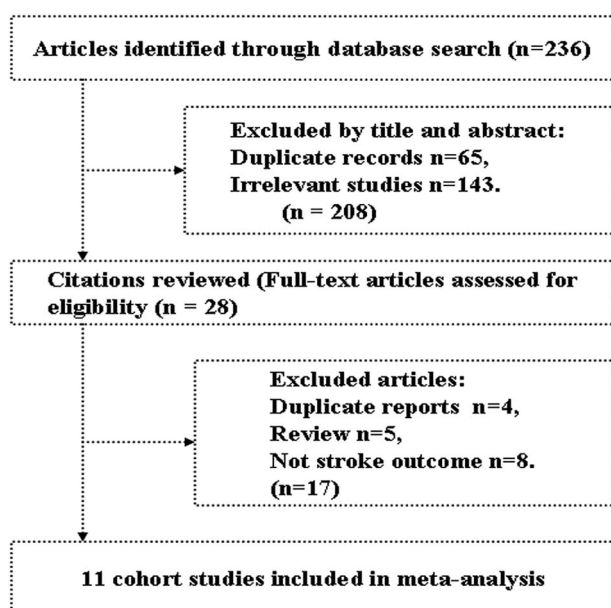
Table 1 Characteristics of studies included in the meta-analysis

First author, publication year	Country/ population	No. of participants (% male)	Age range or mean (years)	Follow-up duration (years)	Assessment of dietary data	Stroke ascertainment	Type and no. of stroke cases	Pre-stroke excluded	Study quality
Hirvonen et al,[8] 2000	Finland/ European	26 497 (100)	50–69	6.1	A self-administered, modified diet history method	Any type of stroke based on ICD-8 or ICD-9 codes	Ischaemic: 736	Yes	8
Arts et al,[9] 2001	The Netherlands/ European	806 (100)	65–84	15	A cross-checked dietary history method	Any type of stroke based on ICD-9 codes	Fatal: 47, Non-fatal: 88	No	7
Knekt et al,[10] 2002	Finland/ European	9131 (NA)	30–69	28	A dietary history interview	Any type of stroke based on ICD-8 codes	Fatal or non-fatal: 681	No	8
Sesso et al,[11] 2003	USA	38 445 (0)	53.9	6.9	A food-frequency questionnaire	Any type of stroke based on clinical diagnosis	Fatal or non-fatal: NA	Yes	9
Marniemi et al,[12] 2005	Finland/ European	755 (47.8)	65–99	10	A dietary history interview	Any type of stroke based on ICD-9 codes	Fatal: 45, Non-fatal: 25	Yes	8
van der Schouw et al,[13] 2005	The Netherlands/ European	16 165 (0)	49–70	6.3	A validated food-frequency questionnaire	Any type of stroke based on ICD-9 codes	Fatal or non-fatal: 147	Yes	9
Mink et al,[14] 2007	USA	34 489 (0)	55–69	16	A food-frequency questionnaire	Any type of stroke based on ICD-9 codes	Fatal: 469	Yes	9
Mursu et al,[15] 2008	Finland/ European	1950 (100)	42–60	15.2	An instructed 4-day food recording by household measures	Any type of stroke based on ICD-9 or ICD-10 codes	Ischaemic: 102	Yes	9
Cassidy et al,[16] 2012	USA	69 622 (0)	30–55	14	A semiquantitative food-frequency questionnaire interview	Any type of stroke based on medical records, autopsy reports and death certificates	Ischaemic: 943	Yes	8
McCullough et al,[17] 2012	USA	98 469 (38.8)	70	7	A semiquantitative food-frequency questionnaire interview	Any type of stroke based on ICD-9 or ICD-10 codes	Fatal: 573	Yes	9
Talaei et al,[18] 2014	Singapore/ Asian	60 298 (44.5)	45–74	14.7	A semiquantitative food-frequency questionnaire interview	Any type of stroke based on ICD-9 codes	Fatal: 1298	No	8

ICD, International Classification of Diseases; NA, not available.

Table 2 Stratified analyses of flavonoid intake and stroke risk

Group	No. of studies	RR (95% CI)	Heterogeneity test			p Value of pooled effect
			χ^2	p Value	I^2, %	
Overall studies						
Fatal/non-fatal stroke	11	0.89 (0.82 to 0.97)	6.48	0.774	0	0.006
Ischaemic stroke	3	0.93 (0.80 to 1.07)	1.03	0.609	0	0.301
Geographical area						
USA	4	0.86 (0.76 to 0.98)	1.50	0.683	0	0.026
Asia	1	0.97 (0.81 to 1.16)	0	–	–	0.740
Europe	6	0.88 (0.77 to 1.00)	3.81	0.577	0	0.051
History of stroke						
Yes	3	0.89 (0.78 to 1.02)	2.10	0.351	4.6	0.105
No	8	0.89 (0.80 to 0.99)	4.38	0.735	0	0.027
Sex						
Male	4	0.82 (0.64 to 1.06)	4.90	0.179	38.8	0.130
Female	5	0.92 (0.80 to 1.05)	2.39	0.664	0	0.216
Combined	3	0.86 (0.72 to 1.04)	2.97	0.227	32.6	0.114
Mean follow-up, years						
≤10	5	0.88 (0.77 to 1.01)	3.78	0.437	0	0.067
>10	6	0.89 (0.81 to 0.99)	2.68	0.750	0	0.036
Quality score						
High, score >8	5	0.85 (0.73 to 0.99)	2.26	0.688	0	0.038
Low, score ≤8	6	0.91 (0.82 to 1.00)	3.73	0.589	0	0.048

RR, relative risk.

Meta regression

In this meta-analysis, although no heterogeneity was observed among individual studies, a multivariate regression was conducted to explore the potential sources of heterogeneity under stroke. The results of regression suggested that stroke outcomes, follow-up duration, sex, geographical area, history of stroke and study quality were not significant sources of heterogeneity.

DISCUSSION

Our meta-analysis of 11 prospective studies indicates that dietary flavonoid intake may be inversely associated with

risk of stroke. In addition, dose-response analysis indicated that a 100 mg/day increment in flavonoid intake was associated with a 9% lower risk of stroke (RR 0.91, 95% CI 0.77 to 1.08). However, this association was not statistically significant.

Potential benefits of flavonoids

Flavonoids, a large class of polyphenols, are widely distributed in plants and are present in considerable amounts in fruits, vegetables, tea and red wine. These bioactive polyphenols are non-energy, non-nutrient secondary metabolites present in plants and cannot be

Figure 2 Random-effects analysis of fully adjusted studies for the highest versus lowest intake of flavonoids and risk of stroke.
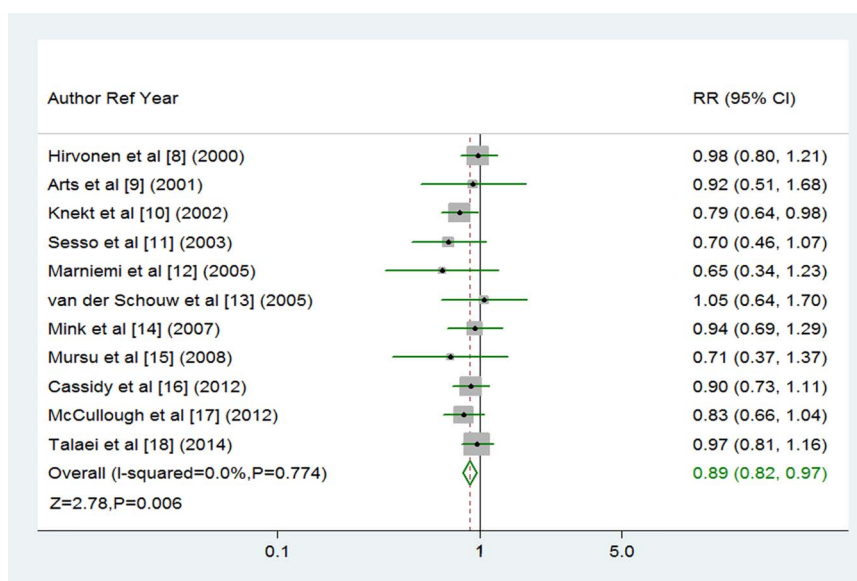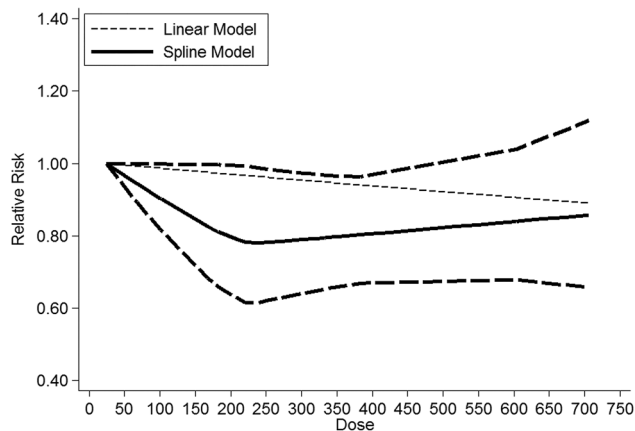
Figure 3 Dose-response relationship between dietary flavonoid intake and stroke risk. The solid line represents point estimates of the association between flavonoid intake and stroke risk, and the dotted lines are 95% CIs. The horizontal line is the reference line.

synthesised by humans.[36] In recent decades, overwhelming evidence has indicated that flavonoid intake is associated with well-known risk-factors for CVD. There is also some evidence that flavonoids may have a role in preventing the development of CVD (eg, coronary heart disease, stroke).[37] [38] In addition, although there are many putative biological mechanisms underlying a possible cardioprotective role for flavonoids,[39] including anti-inflammatory[40] and antioxidant effects,[6] recovery of endothelial function by some compounds[41] and reductions in platelet aggregation,[42] and the effect of individual compounds or interactions between flavonoids is still

largely unknown, flavonol and isoflavone intake may explain some of this beneficial effect.[19 43]

Although the exact mechanism by which flavonoid intake may protect against the development of stroke is still ambiguous, a possible mechanism is the regulation of blood pressure, especially by anthocyanins, due to an increase in endothelial-derived nitric oxide (NO).[44] Moreover, mainly attributed to antioxidant mechanisms, flavonoids reduced mitochondrial lipid peroxidation and loss of mitochondrial transmembrane electric potential caused by oxidative stress induced by ADP plus iron.[45] A third mechanism is the protective action of flavonoids on the anti-inflammatory responses in the brain, which may be attributable to lipid raft disruption and antioxidant effects.[46] Because the flavonoids are very diverse regarding their bioavailability and bioactivity, the rationale for assuming that intakes of all flavonoid classes might have effects on stroke needs more consideration.

Results in relation to other studies

Over the past decades, despite extensive studies that investigated the role of flavonoid intake on cardiovascular disease or cerebrovascular disease, it remains unclear whether the association between flavonoid intake and risk of stroke is causal. One study suggested that individuals with higher flavonoid intake had a lower risk of stroke,[10] although others failed to find an association.[8 9] More, importantly, the possibility of reverse causality should be addressed. A previous meta-analysis of flavonol intake and risk of stroke showed that flavonol intake was inversely associated with stroke incidence.[38] That meta-analysis included
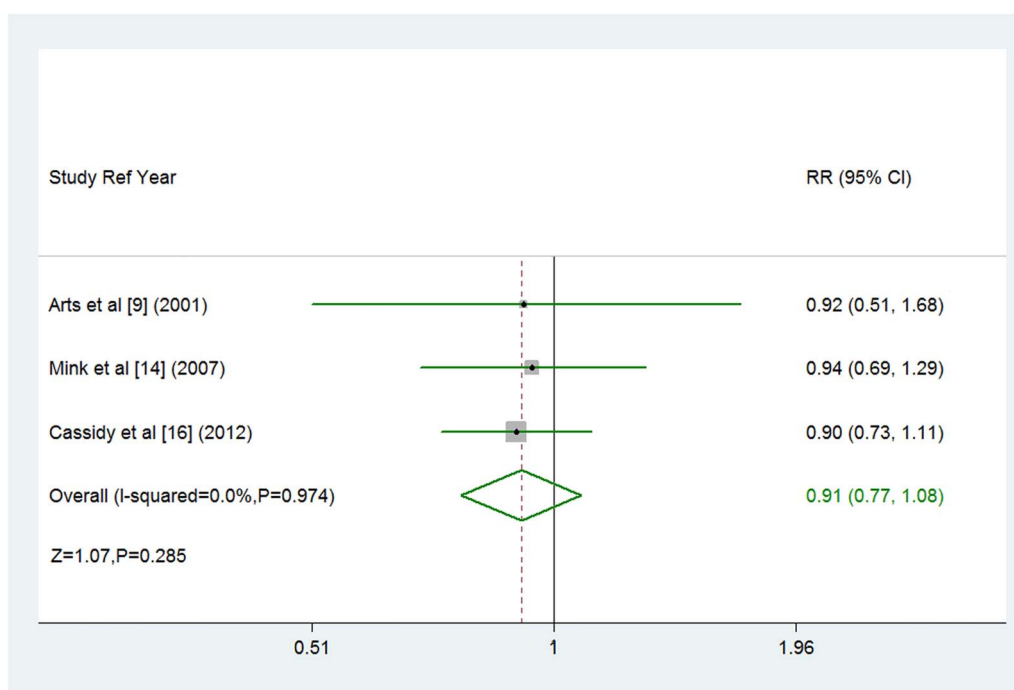
Figure 4 Forest plot of flavonoid intake and risk of stroke. Three cohort studies were eligible for the dose-response analysis of flavonoid intake and risk of stroke. Relative risk <1.0 favours exposure to greater intake of flavonoid.

six prospective cohort studies from three different countries: four of the studies were conducted in Europe (three in Finland and one in The Netherlands) and two in the USA. Inverse associations between flavonol intake and stroke were observed in two prospective cohort studies (the Zutphen Elderly Study and Kuopio Study) and significant inverse associations were found in the Finnish Mobile Clinic Study and Women's Health Study, which result was consistent with a detailed review by Wang et al.[5] Conversely, no associations were noted in the Iowa Women's Health Study and the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study. The results for flavonols were consistent with our findings for flavonoids. However, the data from studies included in the previous meta-analysis were limited to articles published before August 2009.[38] Since then, three new studies on the relationship between flavonoid intake and risk of stroke have been published.[16–18] To obtain a more comprehensive estimate of the putative influence of flavonoids on stroke, we conducted a meta-analysis of prospective cohort studies. To our knowledge, this meta-analysis is the largest to reveal a potential relationship between dietary flavonoid intake and risk of stroke. However, most studies assess dietary flavonoid intake based on self-reported questionnaires using FFQs, and medical records were not always available for stroke classification. Consequently, misclassification of flavonoid intake or stroke was inevitable and may have biased true associations among individual studies.

Strengths and limitations
This study has several important strengths compared with previous meta-analyses.[19] [38] The present meta-analysis included twice as many participants and twice as many stroke cases. To our knowledge, this is the largest meta-analysis to date on flavonoid intake and risk of stroke. We also explored possible sources of heterogeneity using subgroup analyses and the meta-regression method, none of which were significant. Moreover, the presence of a dose-response relationship further strengthened the association of dietary flavonoid intake with risk of stroke. The results should be reliable.

In interpreting the results, some limitations of this meta-analysis should be acknowledged. First, one limitation of any meta-analysis of observational studies is that residual confounding or confounding by unmeasured factors (such as intake of other nutrients) may have affected the strength of the association between flavonoid intake and stroke risk. Additionally, none of the studies used in this dose-response analysis included complete data on total flavonoid intake; the flavonoid intake may have been underestimated in the original studies. Thus, these findings should be treated with caution. Second, we cannot exclude the possibility of recall bias in dietary assessment based on the FFQs. However, the prospective study design and exclusion of participants with chronic diseases at baseline should minimise such bias. Third, a noticeable limitation of our study was the

potential for bias due to inevitable measurement error and misclassification, especially for individuals with lower consumption levels. We attempted to reduce measurement error by adjusting for energy or fibre intake and using cumulatively averaged intake levels. Fourth, we tried to study the main sources of flavonoids but failed due to insufficient data. Different flavonoid subclasses were used in the primary studies included in this meta-analysis. Among the 11 studies, four included the same subclasses (including quercetin, kaempferol, myricetin, luteolin and apigenin),[8] [9] [11] [12] another five studies included similar subclasses (including flavonols, flavones, flavanones, flavan-3-ols, and so on),[10] [14–17] and three studies included isoflavones,[13] [17] [18] as summarised in supplementary table 2. Among the 11 studies, only three were eligible for dose-response analysis. In addition, the flavonoid subclasses differed among the 11 studies: two studies included flavanones, three included flavones, three included flavonols, two included flavan-3-ols, and one included isoflavones. Due to the limited number of studies suitable for dose-response analyses and the insufficient statistical power, we did not conduct dose-response analysis separately on each class. Thus, we evaluated the effects of total flavonoid intake on stroke risk rather than the wide range of flavonoid subclasses. On the one hand, in order to balance the risk of a type I error (the true effect is zero but we reject the null) and a type II error (the true effect is not zero but we fail to reject the null), we combined these estimates using a random-effects model, which takes into account both within-study and between-study variabilities.[24] Thus, these results should be treated with caution. Finally, a possible limitation is due to language bias. We attempted to minimise this bias by searching major electronic databases with no language restriction. However, articles in non-English publications or unpublished reports might not appear in international journal databases and may have been missed in our searches.[47]

CONCLUSIONS
In summary, results from this meta-analysis suggest that higher dietary flavonoid intake may moderately lower the risk of stroke after adjustment for established cardiovascular risk factors. Randomised controlled studies are needed to evaluate the effects of flavonoid intake on stroke risk.

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