Dietary Flavonol Intake May Lower Stroke Risk in Men and Women

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Abstract

Flavonols are strong antioxidants in plant foods and tea is a major dietary source. There is evidence from prospective cohort studies that tea and flavonols are inversely related to stroke incidence. We conducted a metaanalysis of prospective cohort studies to assess quantitatively the strength of the association between flavonol intake and stroke incidence. Prospective cohort studies with data from individuals free of cardiovascular diseases (CVD) or stroke at baseline were included in the metaanalysis. Persons were followed for between 6 and 28 y. Data from 6 cohorts involving 111,067 persons with at least 2155 nonfatal and fatal cases were pooled. A random effects model was used. In all studies included, adjustments were made for major CVD risk factors except for 2 that did not adjust for alcohol and energy intake. A high intake of flavonols compared with a low intake was inversely associated with nonfatal and fatal stroke with a pooled relative risk of 0.80 (95% CI: 0.65, 0.98). Visual inspection of Begg’s funnel plot and Egger’s test ($P = 0.01$) indicated potential publication bias. We conclude that flavonols may reduce stroke risk. J. Nutr. 140: 600–604, 2010.

Introduction

Inverse associations between consumption of foods rich in polyphenols (such as tea, wine, and chocolate or cocoa) and cardiovascular diseases (CVD) were found in various populations (1–5). Recently, data from 10 mainly prospective studies on the association between green and black tea and stroke were evaluated in a metaanalysis and showed that consumption of ≥3 cups/d of tea (green or black) reduced the risk of stroke by 21% (6).

Polyphenols are characterized by their inherent potent antioxidant effects due to the phenolic group(s) and their abundant presence in virtually all plant foods. Many cellular as well as animal studies showed that polyphenols have a range of biochemical properties, such as antioxidant, antiinflammatory, vasodilating, and antithrombotic effects that may explain beneficial effects on CVD (7,8). Polyphenols are a large group of thousands of related compounds belonging to different classes of chemical structures but with the antioxidant phenolic group as their common element. Flavonoids belong to one of these classes and can be subdivided into flavonols, flavones, flavan-3-ols, flavanones, anthocyanins, and isoflavones (9). Dietary intake of all flavonoids may amount to ~500 mg/d (4,10) but is highly dependent on the habitual dietary pattern.

In a comprehensive metaanalysis of 133 randomized controlled trials, it was shown that flavonoid-rich products have a beneficial effect on some intermediate markers for CVD, such as endothelial function, blood pressure, and LDL cholesterol (11). Not surprisingly, no hard endpoints were included in these trials. In addition, these clinical trials were almost exclusively performed with flavonoid-rich foods or -rich extracts containing many other bioactive compounds. So, it is still uncertain which flavonoids are important or whether flavonoids play a role at all (12).

Most prospective cohort studies with individual polyphenols studied the 3 major flavonols in the diet: quercetin, kaempferol, and myricetin. The evidence for an association between the intake of these flavonols and coronary heart disease mortality was summarized in a metaanalysis of prospective cohort studies and the pooled relative risk (RR) was 0.80 (95% CI: 0.69, 0.93) (13). In this paper, we present the first metaanalysis, to our knowledge, of the associations of flavonol intake with stroke incidence.

Methods

Data sources. We searched the PubMed database (U.S. National Library of Medicine and the NIH) for prospective cohort studies published in English from March 1996, the date of the first cohort study on flavonol intake and stroke (14), to August 2009. We used various combinations of the following keywords: polyphenols, phenolics, (bio) flavonoids, flavonols, flavones, quercetin, kaempferol, myricetin, iso-rhamnetin, apigenin, luteolin, cerebrovascular disease, stroke, ischemic stroke, hemorrhagic stroke, cardiovascular diseases, and CVD. We searched references from the publications obtained for additional references and retrieved new publications. This process was repeated until we found no additional references. We identified 7 publications. Two publications were found of the Iowa Women’s Health Study that reported on the same disease outcome (4,15). Only the publication with the longest follow-up period was included in the metaanalysis (4). Six
independent cohort studies were included in the metaanalysis (4,10,14,16–18) (Table 1). Five of the 6 studies used nonfatal and fatal stroke combined as endpoint and 1 used fatal stroke only (4). All studies excluded prevalent cases of CVD or stroke in their analysis.

**Determination of flavonol intake.** We intended to use the sum of flavonols as an estimate, or, if this information was not available, quercetin (the major contributor to flavonol intake). However, most studies used the sum of 3 flavonoids, viz. quercetin, kaempferol, myricetin, apigenin, and luteolin, to estimate the dietary exposure (Table 1). Apigenin and luteolin do not belong to the flavonoid subclass of flavonols but to the very closely related subclass of flavones. However, the intake of flavones is low relative to flavonols, only 7% (19) or even less (20). Flavonol and flavone contents of the foods were extracted from the Dutch database (21,22) and the USDA flavonoid database, which also incorporates the Dutch data (23). These data were obtained with similar analytical methods (24).

To summarize, 1 study analyzed individual flavonols separately and from that study the estimate for quercetin was used (17). Two studies used the sum of the major 3 flavonols: quercetin, kaempferol, and myricetin (4,10). Four studies were considered in which the sum of 3 flavonols and 2 flavones was used as exposure measure (14–16,18).

**Data extraction and data analysis.** Risk estimates and study characteristics were extracted from included papers and double-checked. In all studies, adjustments were made for risk factors and dietary factors. When several estimates for the same exposure were reported with a different level of adjustment, the most fully adjusted estimate was chosen. Risk estimates and SE were converted to a log-scale for use in the metaanalysis.

We performed metaanalysis of risk estimates comparing the highest category of flavonol exposure with the lowest category. We applied a random effects model. This model takes into account and weighs study effects according to both study sample size and between-study variation (25). We quantified the extent of heterogeneity by using $I^2$, the percentage of total variation across studies attributable to heterogeneity between studies rather than chance (26). $I^2$ values of 25–50% were considered low, 50–75% moderate, and >75% high (26). We constructed Begg’s funnel plots and used Egger’s test to detect small study bias. Sensitivity analyses were carried out by excluding selected studies and reanalyzing the data. All statistical analyses were conducted using the statistical package Stata (SE version 8.0). Values in the text are RR (95% CI).

**Results**

**Exposure to flavonols.** Dietary intake was assessed at baseline. FFQ were applied in 3 studies (4,16,18) (Table 2). In the other studies, more extensive dietary survey methods were used, such as cross-check dietary history (14,17) and 4-d food records (10). In one-half of the studies, flavonols were included in the flavonol intake (Table 2). Comparisons of high and low flavonol intake were based on quintiles in all US cohorts and in the Finnish ATBC study, whereas the other European cohorts used quartiles or tertiles (Table 2). Flavonol intakes were quite similar in the different cohorts, with the exception of the Finnish cohorts. Intakes in these cohorts were at least 50% lower (10,16,17).

Tea probably was the major flavonol source in all cohorts, but its contribution differed among countries. However, dietary sources were poorly specified in the Finnish cohorts. In a typical tea-drinking country like The Netherlands, tea contributed 70% of the flavonol intake (Table 2). In the US, tea contributed ~30%. Onions and apples were other important sources, with contributions between 25 and 8%.

**Nonfatal and fatal stroke.** The 6 cohorts from 3 different countries, The Netherlands, Finland, and the United States, included 111,067 persons with at least 2155 fatal and nonfatal cases of stroke. The exact number of cases is unknown, because

### TABLE 1

Prospective cohort studies on flavonols (and flavones) and stroke incidence in persons free of CVD at baseline

<table>
<thead>
<tr>
<th>Cohort</th>
<th>Population and age</th>
<th>Flavonols</th>
<th>Follow-up period, y</th>
<th>Endpoint</th>
<th>Cases, n</th>
<th>Adjusted RR (95% CI)</th>
<th>Adjusted covariates</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zutphen (NL)</td>
<td>552 M, 50–69 y</td>
<td>Flavonols</td>
<td>15</td>
<td>Nonfatal + fatal stroke</td>
<td>42</td>
<td>0.27 (0.11, 0.70)</td>
<td>Blood lipids, intake of alcohol, energy, and fish</td>
<td>Keli (14)</td>
</tr>
<tr>
<td>ATBC (Fin)</td>
<td>26497 M, 50–69 y</td>
<td>Flavonols</td>
<td>6.1</td>
<td>Nonfatal + fatal ischemic stroke</td>
<td>736</td>
<td>0.98 (0.80, 1.21)</td>
<td>Blood lipids, diabetes, education, intake of alcohol, and supplements</td>
<td>Hirvonen (16)</td>
</tr>
<tr>
<td>Finnish Mobile</td>
<td>9131 MF, 39.3 ± 15.8 y</td>
<td>Quercetin</td>
<td>28</td>
<td>Nonfatal + fatal stroke</td>
<td>806</td>
<td>0.86 (0.70, 1.05)</td>
<td>Blood lipids, diabetes, occupation</td>
<td>Knekt (17)</td>
</tr>
<tr>
<td>Kuopio IHD (Fin)</td>
<td>1950 M, 52.4 ± 5.3 y</td>
<td>Flavonols</td>
<td>15.2</td>
<td>Nonfatal + fatal ischemic stroke</td>
<td>102</td>
<td>0.55 (0.31, 0.99)</td>
<td>Blood lipids, diabetes, CVD in family, hypertension medication, intake of alcohol, folate, vitamin E, total fat, and saturated fat</td>
<td>Mursu (10)</td>
</tr>
<tr>
<td>Womens Health (US)</td>
<td>38,445 F, 53.9 ± 7.0 y</td>
<td>Flavonols</td>
<td>6.9</td>
<td>Nonfatal + fatal stroke</td>
<td>?</td>
<td>0.70 (0.46, 1.07)</td>
<td>Blood lipids, diabetes, myocardial infarct in family, exercise, estrogen and aspirin use, intake of alcohol, vitamin E, and β-carotene</td>
<td>Sesso (18)</td>
</tr>
<tr>
<td>Iowa Womens Health (US)</td>
<td>34,492 F, 61.5 ± 4.2 y</td>
<td>Flavonols</td>
<td>10</td>
<td>Fatal stroke</td>
<td>131</td>
<td>1.18 (0.70, 2.00)</td>
<td>Blood lipids, intake of alcohol, vitamin E, and aspirin use, intake of alcohol, vitamin E, and β-carotene</td>
<td>Yochum (15)</td>
</tr>
<tr>
<td>Iowa Womens Health (US)</td>
<td>34,492 F, 61.5 ± 4.2 y</td>
<td>Flavonols</td>
<td>16</td>
<td>Fatal stroke</td>
<td>469</td>
<td>0.92 (0.67, 1.24)</td>
<td>Waist:hip ratio, marital status, education, physical activity, estrogen use, intake of energy</td>
<td>Mink (4)</td>
</tr>
</tbody>
</table>

1 Age: range, median, or mean ± SD. M, Male; F, female.
2 Flavonols = sum of quercetin, kaempferol, and myricetin; flavones = sum of luteolin and apigenin.
3 High-low categories are given in Table 2.
4 All studies were adjusted for age, gender (where appropriate), BMI, smoking, and blood pressure. Additional adjustments are shown for each study.
5 Not included in the metaanalysis.
1 cohort (18) did not report the number of cases. Persons were followed for between 6 and 28 y. Three of the studies included only men (10,14,16) and 2 only women (4,18). One study presented only the risk estimates for males and females combined (17). Stroke endpoints were either not specified or not analyzed separately and were combined ischemic and hemorrhagic stroke, except 2 studies (10,16) for which ischemic stroke data were used (Table 1). In the Finnish Mobile Clinic study (17), the RR for ischemic stroke (0.80; 95% CI: 0.60, 1.05) and total stroke (0.86; 95% CI: 0.70, 1.05) were similar.

In all studies, adjustments were made for age, gender, BMI, smoking, blood pressure, and for blood lipids in all but 1 (4). Adjustments for dietary factors, mostly energy and alcohol, were made in all studies except 2 (17,27). The 6 estimates varied from 0.27 (95% CI: 0.11, 0.68) (14) to 0.98 (95% CI: 0.80, 1.21) (16); smaller studies showed stronger effects than the larger ones. The pooled RR was 0.80 (95% CI: 0.65, 0.98) (Fig. 1). Heterogeneity of the studies was moderate (54%) and borderline significant (P = 0.05). The asymmetry in the Begg’s funnel plot (Fig. 2) suggests that smaller studies with small effects may not have been published. In addition, Egger’s test showed evidence of a possible publication bias (P = 0.01).

**Discussion**

This metaanalysis showed that a high intake of flavonols compared with a low intake was associated with a 20% lower risk of stroke incidence. Because of the small number of studies and an indication for publication bias, this result should be interpreted with caution.

![FIGURE 1](https://academic.oup.com/jn/article-abstract/140/3/600/4689169) Forest plot of the RR of fatal and nonfatal stroke and flavonol consumption (highest compared with lowest category of intake) for 6 independent estimates from 6 cohort studies. The contribution of each estimate to the metaanalysis (its weight) is represented by the size of the black box. Heterogeneity $I^2 = 54\%$ ($P = 0.05$). M, Male; F, female.
FIGURE 2  Begg’s funnel plot (6 estimates from 6 cohort studies) with pseudo-95% confidence limits for the RR of fatal and nonfatal stroke and flavonol consumption (highest compared with lowest category of intake).

Our results are in agreement with those of the metaanalysis on tea consumption and the risk of stroke (6) and suggest that flavonols might be the protective compounds in tea. Three out of 6 of the cohort studies were included in both metaanalyses, which could indicate that flavonols are a proxy for tea consumption. However, in the Finnish studies and to a lesser extent also in the U.S. studies, tea was not the major dietary source of flavonols. Interestingly, RR for green and black tea were similar in the metaanalysis on tea and stroke. This points to compounds with comparable contents in green and black tea. Flavonols are the only flavonoids that do not differ between green and black tea, whereas, for instance, the concentration of the more abundant monomeric flavan-3-ols (e.g. epigallocatechin gallate) in black tea is only 20% of that in green tea (23). Thus, flavonols might explain the inverse association between tea consumption and stroke.

Our pooled analysis of 6 prospective cohort studies showed a lower risk of stroke incidence at a high intake of flavonols (RR = 0.80; 95% CI: 0.65, 0.98). Although all studies had a RR < 1, there was a large range in RR. By far the lowest (0.27) was observed in the Dutch cohort of Zutphen (14). An explanation for this strong association could be that in this study, detailed food consumption data were collected by the cross-check dietary history method. Also, the average flavonol intake was calculated based on 3 estimates of food consumption separated by 5 y. This reduced the measurement error in the exposure. However, we cannot rule out that chance also played a role because of the small size of this cohort study.

Publication bias is a potential threat to the validity of metaanalyses, because small studies with small effects may have been published less than small studies with large effects (28). Two commonly used tests to address publication bias are Begg’s and Egger’s tests. Although they may provide useful information, some limitations should be mentioned. The tests (but also the funnel plot) require a reasonable amount of dispersion in the sample sizes and a reasonable number of studies, and the tests tend to have low power. Therefore, we gave more emphasis to an informal visual inspection of the funnel plot. Negative, small studies would be expected to fall in the upper right quadrant of this plot. There was no study in this area that suggested visual evidence of publication bias; this was confirmed by Egger’s test. If it is indeed the case that small studies with small effects have not been published, the strength of this association might be an overestimate of the true association between flavonol intake and stroke.

Residual confounding might also contribute to the inverse associations observed, because it has been reported that the intake of flavonols was positively associated with a healthy lifestyle. Persons with higher intakes of flavonols or tea were more likely to be nonsmokers and had lower intakes of total and saturated fat, lower BMI, and more education (29–31). Despite extensive adjustments for risk factors in all studies, we cannot rule out that part of the observed lower risk might be due to a healthy lifestyle. Residual confounding by other bioactive compounds present in tea and onions, the major sources of flavonols, cannot be ruled out either.

Exposure to flavonols was in one-half of the studies measured with semiquantitative FFQ. It is questionable whether all FFQ did assess intake of onions, the second-most important dietary flavonol source, in sufficient detail, because onions are mostly hidden ingredients in sauces and soups (20). So flavonol intake may have been underestimated (4). The validity of these FFQ for assessing flavonols has to be investigated.

One-half of the studies used the sum of flavonols and flavones as an exposure measure. Flavones are closely related to flavonols; they lack only the 3-OH in the C-ring, but the contribution of flavones is quite small (19,20). So, it is likely that the total intake of flavonols and flavones approximates flavonol exposure quite well. Quercetin is by far the major dietary flavonol and contributes ~70% of all flavonols in the diet (19). Thus, in essence, these cohort studies likely estimated the beneficial effects of quercetin.

The very low flavonol intake in the Finnish Mobile Clinic study (17), even compared with the other Finnish cohorts, is likely due to the fact that tea consumption was not estimated. Hirvensen (27) showed that tea consumption in the Finnish ATBC cohort was generally <1 cup (170 mL/d), accounting for ~6 mg flavonols/d. Consequently, real flavonol intake is quite comparable in the Finnish cohorts. The intake of flavonols in Finland was at the time of the baseline surveys in the 1970s and 1980s much lower than that in The Netherlands and US (Table 2).

Tea was the major flavonol source in the Dutch cohort, whereas in the U.S. cohorts, besides tea, onions, apples, and broccoli were also important. This is relevant for plasma and tissue levels of flavonols, because the bioavailability of flavonols in onions is much better than that of tea and apples (32). As a consequence, plasma and tissue concentrations of flavonols depend also on the type of dietary flavonol source. In the etiology of stroke, these plasma and tissue concentrations are relevant. Flavonol bioavailability determines the relation between flavonols consumed and plasma and tissue levels. Flavonol bioavailability of a food is dependent on the type of flavonol glycoside in that food (32). The food composition databases used in the current studies did not specify these glycosides and, consequently, bioavailability could not be accounted for.

In summary, we showed for the first time, to our knowledge, that flavonol intake was inversely associated with stroke incidence. In a previous metaanalysis, flavonols were inversely associated with coronary heart disease mortality (13). A protective effect of the major flavonol quercetin on intermediate CVD outcomes has been shown in 2 randomized, placebo-controlled clinical trials: quercetin increased the plasma pool of NO by 35% and reduced plasma endothelin-1 by 7%, thus enhancing endothelial function (33), and in hypertensive subjects, quercetin reduced systolic blood pressure by 7 mm Hg (34). We conclude that evidence is accumulating that flavonol intake is inversely related to different CVD outcomes.
Acknowledgments
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Literature Cited