PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Dietary flavonoid intake and the risk of stroke: A dose-response meta-analysis of prospective cohort studies
AUTHORS	Tang, Zhenyu; Li, Min; Zhang, Xiaowei; Hou, Wenshang

VERSION 1 - REVIEW

REVIEWER	Julia Peterson Tufts University USA
REVIEW RETURNED	26-Jun-2015

GENERAL COMMENTS	There is a problem with conflating all these studies together and using total flavonoids. Only 3 studies involved 5 or more classes of flavonoids. Please add the flavonoid classes for each paper to Table 1. A second problem is assuming that sources of flavonoid are all the same. Some classes are found predominately in one (or sometimes two) family [flavanones in Citrus, isoflavones in Fabaceae (Leguminosae)]. Lastly flavonoids is not used as an adjective (flavonoid consumption, flavonoid intake). Flavonoid as an adjective is considered to include all classes and compounds. Other suggested changes are in an attached excel file. The reviewer also provided an Excel with additional comments. Please contact the publisher for full details.

REVIEWER	Arno Greyling Unilever R&D Vlaardingen, The Netherlands Arno Greyling is employed by employed by Unilever R&D Vlaardingen. Unilever produced and markets foods and beverages.
REVIEW RETURNED	20-Jul-2015

GENERAL COMMENTS	This is a well-conducted meta-analysis that provides an up-to-date summary of the available evidence regarding the association between flavonoid intake and stroke risk. A few points need the authors attention: 1) I'm having difficulty in understanding the logic behind the dose-response analysis conducted in this study. Specifically only two of

	the studies (Cassidy et al 2012 & Mink et al 2007) actually calculated total flavonoid intake whilst the other two studies used in the analysis (Talaei et al 2014 & Arts et al 2001) only measured one and at most three of the six flavonoid classes required to calculate total flavonoid intake. How exactly were the intake data from the latter two studies applied in the dose-response analysis? Were the reported intakes of the individual sub-classes (isoflavone and catechin respectively) used as proxies for total intake? If so, this seems to be a fundamental error since "total flavonoid intake" would be underestimated and the effect size like overestimated in these two studies. If the authors adjusted their dose-response analysis in some way to take into account the fact that the studies of Mink and Arts did not include complete data on total flavonoid intake, this should be clearly indicated in their statistical methods section. Likewise, if the Authors made additional assumptions in order to derive an estimate of total flavonoid intake, this should be mentioned and discussed. Specialist statistical review regarding the applicability of the Greenland & Longnecker method in this specific context is recommended. 2) In the discussion it is mentioned that the main sources of flavonoid(classes) were likely the same between studies (page 8, lines 6-7). I would like to draw the Author's attention to recent work (Vogiatzoglou et al 2015, PLoS One. 2015; 10(5): e0128132) suggesting that the sources of flavonoid classes can vary considerably between different populations (at least in Europe). It would be interesting if this observation and its potential impact regarding the current study were discussed. Minor points: Typo on page 4, line 24/25 – "depression" should be replaced with "flavonoid intake"?

REVIEWER	Colin Kay University of East Anglia, UK
REVIEW RETURNED	20-Jul-2015

GENERAL COMMENTS	Overall the article is well written and details the ever expanding base of evidence for the protective effects of flavonoids on vascular disease. The abstract and methodology is appropriately detailed and succinct. With respect 'meta-statistics' I may not be fully qualified to provide detailed feedback. Any flaws in the manuscript are more related to what is missing, rather than what is there. For example, it would be informative if the authors described the following: Search strategy. The literature search strategy seems limited as many classes of flavonoids are missing. Are the authors sure some studies were not excluded by focusing on flavonols and flavones in the search strategy. What was the authors rationale for not including anthocyanins, flavononones and their prominent subclasses such as cyanidin, peonidin, malvidin, delphinidin, apigenin, etc? Data extraction. The supplementary information is quite limited for a meta-analysis, for example, the supplement would benefit from a description of what papers authors were contacted to provide

	additional information and what was this missing information. This is particularly useful for others to take this work forward or for updating the meta-analysis in the future. Results. It states in the results that only 4 of the 10 studies were available for dose-response analysis which is misleading based on the title which suggests the primary analysis is dose-response. I would therefore question the title and use of the term 'dose-response meta-analysis'. Also is this an accepted terminology in systematic reviews or meta-analysis? Based on the description of the work and the presentation of the findings, the study is actually a meta-analysis looking at flavonoids and effect on stroke incidence with a secondary analysis of dose-response. No p value is provided for the non-significant result on line 52 of the results section. There is limited description of the magnitude of the observed risk reduction relative to incidence in the 'real-world'… i.e., what are the implications of such a reduction in risk. There is also limited description of how this level of risk could be attained by dietary modification, given the fact that the authors claim it's a dose-response study of dietary components (i.e., what dose, what risk, how would this be achieved through dietary means). There is limited description of actual dose range, i.e., what was the common high vs low versus median dose? Limited discussion on power of study relative to other meta-analysis in the flavonoid field. i.e., is 10 studies large or small relatively? Is the population (n=) large or small relative to other studies in the field? Discussion. There is no discussion on the relevance of the 100mg dose increment. i.e., what foods and how much would you need to provide such a dose? What were the major dietary sources of flavonoids reported in the studies? How was dose established from diet records? Was a database used to establish flavonoid concentration in these studies? On line 47 of the discussion why did the authors highlight flavonols and isoflavones, just two subclasses of flavonoid? This does not seem necessary or justified based on the design of the study and search terms. The discussion on mechanism of action seems out of place and the mechanisms are not linked to stroke in the text. Particularly as cohort studies tell us little about mechanism. There is limited description of other potential confounders in the diet such as total fruit and vegetable intake. Figures. Limited description in legend of figure 3, such as what does each line represent?

REVIEWER	Bernet Kato New England Research Institutes (Massachusetts), United States of America
REVIEW RETURNED	10-Oct-2015

GENERAL COMMENTS	**Dietary flavonoids intake and the risk of stroke: A dose-**

response meta-analysis of prospective cohort studies

General Comments

The paper reports the results of a meta-analysis and systematic review to evaluate the potential association between the intake of flavonoids and risk of stroke. Systematic reviews of the best available evidence regarding the benefits of interventions can inform decision making in clinical practice and public health. The results are therefore of interest in public health practice.

Next I present comments on each section of the paper.

Article Summary

I suggest that the 'strengths' and 'limitations' of the study be presented separately.

Statistical analysis

1. It is mentioned that "The relative risks (RR) were used as the common measure between depression and stroke, and the hazard ratios (HRs) were considered equivalent to RRs. Data analysis used multivariate outcome data".

   The study was looking at association between intake of flavonoids and risk of stroke. It is not clear how the issues of depression and hazard ratios come in. Furthermore, the authors should clarify what is meant by "data analysis used multivariate outcome data".

Results

1. Under literature search it is mentioned that out of the 11 studies excluded; four studies had no stroke outcomes, 3 were duplicate reports and 4 studies were excluded due to "review". The authors should clarify what is meant by excluding studies due to 'review'.
2. The authors note that the dietary assessment flavonoids intake varied across studies, with most studies measuring intake using food frequency questionnaires and dietary history. In order for the readers to get a better feel of what the relative risks estimates (and their confidence intervals) from the 10 studies used in the meta-analysis mean, it would be helpful to mention how flavonoids intake was measured in each of the 10 studies. For example was intake measured in mg/day or mg per week, etc.?

3. Table 2 (Stratified analyses of flavonoids intake and stroke risk) – the first group analysis titled "Overall studies" suggests that there were 10 studies categorized as Fatal/nonfatal stroke and 3 studies categorized as Ischemic

	stroke. However, the flow chart on page 15 suggests that 10 studies were included in the meta-analysis. Does this imply that the 'Ischemic stroke' group is a subset of the 'Fatal/nonfatal stroke' group? 4. Figures 3 (dose response relationship between dietary flavonoids intake and stroke risk) and 4 (Forest plot of flavonoids and risk of stroke) are hardly discussed in the text. a. What do the different lines in figure 3 represent and what should the reader learn from the figure? b. Only 4 out of the 10 studies were eligible for the dose-response analysis. What were the criteria for eligibility? **Discussion** Page 7: Limitation 1: I suggest you change the sentence to read "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 association between flavonoids intake and stroke risk". Thus these findings should be treated with caution. Page 8: Limitation 4 states that "we could not study the main sources of flavonoids because of the insufficient data". The authors should clarify what they mean by this. **Edits** The following sentences have typos/grammatical errors which should be corrected. 1. Page 4: Assessment of study quality. The last sentence that reads "Studies were graded as the high-quality if they met >8 awarded stars. 2. Page 4: Fourth sentence from the bottom - "All available data were conducted in the primary analysis". 3. Page 5: Third sentence from top – separate the words 'above' and 'mentioned'. 4. Page 6: Third sentence from top – the upper confidence limit should be 1 rather than 100. 5. Page 7: Strengths and limitations. The sentence that begins with 'We'. 6. Page 12: Table 1, column 4 - the word range is misspelt.

VERSION 1 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name
Julia Peterson
Institution and Country
Tufts University
USA
Q1: There is a problem with conflating all these studies together and using total flavonoids. Only 3

studies involved 5 or more classes of flavonoids. Please add the flavonoid classes for each paper to Table 1.
R1: Once again, thank you for your careful review. The flavonoid subclasses and compounds have been added as you suggested (supplemental table 2).

Q2: A second problem is assuming that sources of flavonoid are all the same. Some classes are found predominately in one (or sometimes two) family [flavanones in Citrus, isoflavones in Fabaceae (Leguminosae)].
R2: We are very sorry that we did not explain this critical point clearly. In fact, we tried to study the main sources of flavonoids. But, we failed it because of the insufficient data. Different sub-classes for flavonoid were used in the primary studies included in this meta-analysis. Among 10 studies, 4 studies included the same sub-classes (including quercetin, kaempherol, myricetin, luteolin, and apigenin),8,9,11,12 another 4 studies included the similar sub-classes (including flavonols, flavones, flavanones, flavan-3-ols, and so on),10,14-16 and 2 studies included the similar compounds (including daidzein, genistein, gormononetin, biochanin A, and glycitein).13,17 as summarized in supplemental table 2. Thus, we evaluated the effects of total flavonoid intake on stroke risk rather than the wide-range of flavonoid sub-classes. 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) (Introduction to Meta-Analysis, Michael Borenstein et al.), we combined these estimates using a random-effects model, which takes into account both within-study and between-study variabilities (DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177-88.). Thus, these results should be treated with caution.
Accordingly, we have addressed this important point in the revised manuscript (Page 8).

Q3: Lastly flavonoids is not used as an adjective (flavonoid consumption, flavonoid intake). Flavonoid as an adjective is considered to include all classes and compounds.
R3: Thank you for your careful review and very constructive suggestion. We have made corrections accordingly in the revised manuscript, and all amendments are highlighted in red.

Q4: Other suggested changes are in an attached excel file.
R4: Thank you for your careful review and very constructive suggestion. We have made corrections accordingly in the revised manuscript, and all amendments are highlighted in red.

Reviewer: 2
Reviewer Name
Arno Greyling
Institution and Country
Unilever R&D Vlaardingen,
The Netherlands
Q1: I'm having difficulty in understanding the logic behind the dose-response analysis conducted in this study. Specifically only two of the studies (Cassidy et al 2012 & Mink et al 2007) actually calculated total flavonoid intake whilst the other two studies used in the analysis (Talaei et al 2014 & Arts et al 2001) only measured one and at most three of the six flavonoid classes required to calculate total flavonoid intake. How exactly were the intake data from the latter two studies applied in the dose-response analysis? Were the reported intakes of the individual sub-classes (isoflavone and catechin respectively) used as proxies for total intake? If so, this seems to be a fundamental error since “total flavonoid intake” would be underestimated and the effect size like overestimated in these two studies. If the authors adjusted their dose-response analysis in some way to take into account the fact that the studies of Mink and Arts did not include complete data on total flavonoid intake, this should be clearly indicated in their statistical methods section. Likewise, if the Authors made additional assumptions in order to derive an estimate of total flavonoid intake, this should be mentioned and discussed. Specialist statistical review regarding the applicability of the Greenland & Longnecker method in this

specific context is recommended.
R1: We are very sorry that we did not explain this critical point clearly. Among 4 studies (Arts et al 2001 & Mink et al 2007 & Cassidy et al 2012 & Talaei et al 2014), Mink et al (2007) and Cassidy et al (2012) included the similar sub-classes (including flavonols, flavones, flavanones, flavan-3-ols, and so on),14,16 Talaei et al (2014) included isoflavones, and Arts et al (2001) included flavonol from sources other than tea, as summarized in supplemental table 2. In fact, 4 studies did not include complete data on total flavonoid intake in this dose-response analysis (Vogiatzoglou A, Mulligan AA, Lentjes MA, et al. PLoS One 2015;10:e0128132). When the result on total flavonoid intake in our dose-response meta-analysis was not available, we used data from flavonols, flavones, flavanones, flavan-3-ols, and isoflavones (in the sequential order) as an equivalent to total flavonoid. Thus, the intake of flavonoid may have been underestimated in these original studies and caution should be exercised in extrapolating the results.
Accordingly, we have invited an expert statistical scientist to revise the manuscript. We have also addressed this important point in their statistical methods and discussions section.

Q2: In the discussion it is mentioned that the main sources of flavonoid (classes) were likely the same between studies (page 8, lines 6-7). I would like to draw the Author's attention to recent work (Vogiatzoglou et al 2015, PLoS One. 2015; 10(5): e0128132) suggesting that the sources of flavonoid classes can vary considerably between different populations (at least in Europe). It would be interesting if this observation and its potential impact regarding the current study were discussed.
R2: Thank you for your constructive suggestion. Vogiatzoglou et al provided a detailed overview of flavonoid consumption in European adults, and the results suggested the habitual intake of flavonoids in Europe was below the amounts found to have a significant health effect. The results were therefore of interest in public health practice. The results were consistent with our findings on flavonoids. In our subgroup analysis, the pooled relative risk of stroke was 0.88 (95% confidence interval: 0.77-1.00) in European. We have made corrections accordingly in the revised manuscript, and all amendments are highlighted in red.

Minor points:
Q1: Typo on page 4, line 24/25 – "depression" should be replaced with "flavonoid intake"?
R1: Thank you for your careful review. We have made correction accordingly in the revised manuscript, and this amendment is highlighted in red.

Reviewer: 3
Reviewer Name
Colin Kay
Institution and Country
University of East Anglia, UK
Q1: Search strategy. The literature search strategy seems limited as many classes of flavonoids are missing. Are the authors sure some studies were not excluded by focusing on flavonols and flavones in the search strategy. What was the authors rationale for not including anthocyanins, flavononones and their prominent subclasses such as cyanidin, peonidin, malvidin, delphinidin, apigenin, etc?
R1: We are very sorry that we did not explain this critical point clearly. To some extent, owing to our search strategy, we can not sure some studies were not excluded. But, the search strategy was conducted according to the recommendations of the Meta-analysis of Observational Studies in Epidemiology (MOOSE). On the other hand, two authors (ML and WH) searched there databases and checked them according to the eligible criteria and exclusion criteria. In literature search section, the main sources of flavonoid ("flavonoids," "polyphenols," "phenolics," "flavonols," "flavones," "quercetin," "kaempferol," "myricetin," "isorhamnetin," "apigenin," "luteolin,") were included (Vogiatzoglou A, Mulligan AA, Lentjes MA, et al. PLoS One 2015;10:e0128132.). Additionally, we also combined with the search strategy by Wang et al (Wang X, Ouyang YY, Liu J, et al. Br J Nutr 2014;111:1-11). Finally, the possible limitation is due to language and selective bias. We attempted to minimize this

bias by searching major electronic databases with no language restriction. However, several articles published in non-English, unpublished or negative reports might not appear in international journal databases, and could be omitted by our searches.

Q2: Data extraction. The supplementary information is quite limited for a meta-analysis, for example, the supplement would benefit from a description of what papers authors were contacted to provide additional information and what was this missing information. This is particularly useful for others to take this work forward or for updating the meta-analysis in the future.
R2: We are very sorry that we did not explain this critical point clearly. In fact, we tried to obtain additional relevant articles by scanning conference summaries and reference lists of articles identified in the initial searches and contact authors to obtain additional information for relevant studies. For example, in our dose-response analysis, only 4 studies were included (Arts et al 2001 & Mink et al 2007 & Cassidy et al 2012 & Talaei et al 2014), others (6 studies) did not include available data (the distributions of cases, person years for exposure categories, and median/mean of flavonoid intake level for each comparison group) on total flavonoid intake. We have made corrections accordingly in literature search section, and all amendments are highlighted in red.

Q3: Results. It states in the results that only 4 of the 10 studies were available for dose-response analysis which is misleading based on the title which suggests the primary analysis is dose-response. I would therefore question the title and use of the term 'dose-response meta-analysis'. Also is this an accepted terminology in systematic reviews or meta-analysis? Based on the description of the work and the presentation of the findings, the study is actually a meta-analysis looking at flavonoids and effect on stroke incidence with a secondary analysis of dose-response.
No p value is provided for the non-significant result on line 52 of the results section.
There is limited description of the magnitude of the observed risk reduction relative to incidence in the 'real-world'… i.e., what are the implications of such a reduction in risk. There is also limited description of how this level of risk could be attained by dietary modification, given the fact that the authors claim it's a dose-response study of dietary components (i.e., what dose, what risk, how would this be achieved through dietary means). There is limited description of actual dose range, i.e., what was the common high vs low versus median dose? Limited discussion on power of study relative to other meta-analysis in the flavonoid field. i.e., is 10 studies large or small relatively? Is the population (n=) large or small relative to other studies in the field?
R3: We are very sorry that we did not explain this critical point clearly. In fact, we provided the p value for the non-significant result of the results section (Figure 4). We have made correction accordingly in title, abstract and result sections, and this amendment is highlighted in red. Results from this meta-analysis suggested that higher dietary flavonoid intake may moderately lower the risk of stroke after adjustment of established cardiovascular risk factors (such as Age, BMI, SBP, DBP, height, cholesterol, diabetes, history CHD, smoking, alcohol, supplementation group, and so on), as summarized in supplemental table 1. Thus, the role of flavonoid intake on stroke prevention is important. Compared with the previous meta-analyses, this meta-analysis has several important strengths. The present meta-analysis included 2 times more participants and 2 times more stroke cases. To our knowledge, this is the largest meta-analysis on flavonoid intake and the risk of stroke, as summarized in the discussion section. Additionally, as described in the statistical analysis sections, 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.
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Q4: Discussion. There is no discussion on the relevance of the 100mg dose increment. i.e., what foods and how much would you need to provide such a dose? What were the major dietary sources of flavonoids reported in the studies? How was dose established from diet records? Was a database

used to establish flavonoid concentration in these studies? On line 47 of the discussion why did the authors highlight flavonols and isoflavones, just two subclasses of flavonoid? This does not seem necessary or justified based on the design of the study and search terms. The discussion on mechanism of action seems out of place and the mechanisms are not linked to stroke in the text. Particularly as cohort studies tell us little about mechanism.
There is limited description of other potential confounders in the diet such as total fruit and vegetable intake.
R4: Thank you for your careful review. As summarized in supplemental table 2, all studies did not include complete data on total flavonoid intake in this meta-analysis. But, it was obvious that flavonols and isoflavones were the major dietary sources of flavonoids (kaempferol, quercetin, myricetin, and isorhamnetin). A validated food-frequency questionnaire was used to assess dietary data. The Comprehensive European Food Consumption Database was used to determine mean and median intake of flavonoids. There is no doubt, in interpreting the results, residual confounding should be acknowledged (such as total fruit and vegetable intake). But, as an observational study, residual confounding or confounding by unmeasured factors (such as intake of other nutrients) is inevitable. Thus, we used the multivariate-adjusted relative risk (RR) and 95% confidence interval (CI) for stroke incidence or mortality associated with flavonoids intake to minimize this bias. We have made corrections accordingly in mechanism section, and all amendments are highlighted in red.

Q5: Figures. Limited description in legend of figure 3, such as what does each line represent?
R5: Thank you for your careful review. We have made correction accordingly in figure 3, and this amendment is highlighted in red.

Reviewer: 4
Reviewer Name
Bernet Kato
Institution and Country
New England Research Institutes (Massachusetts), United States of America
Q1: I suggest that the ‘strengths’ and ‘limitations’ of the study be presented separately.
R1: Thank you for your careful review. We have made correction accordingly in the strengths and limitations sections, and this amendment is highlighted in red.

Q2: It is mentioned that “The relative risks (RR) were used as the common measure between depression and stroke, and the hazard ratios (HRs) were considered equivalent to RRs. Data analysis used multivariate outcome data”. The study was looking at association between intake of flavonoids and risk of stroke. It is not clear how the issues of depression and hazard ratios come in. Furthermore, the authors should clarify what is meant by “data analysis used multivariate outcome data”.
R2: Thank you for your careful review. This is our fault, it was not mentioned that the issues of depression. Among 10 studies, some studies used hazards ratios to examine associations between flavonoid intake and stroke risk, so the hazard ratios were considered equivalent to relative risks in this text. In the multivariate model, the original study adjusted for age, sex, cigarette smoking, and so on, it indicated that the pooled estimates remained reliable. We have made correction accordingly in the revised manuscript, and this amendment is highlighted in red.

Q3: Under literature search it is mentioned that out of the 11 studies excluded; four studies had no stroke outcomes, 3 were duplicate reports and 4 studies were excluded due to “review”. The authors should clarify what is meant by excluding studies due to ‘review’.
R3: We are very sorry that we did not explain this critical point clearly. “Review” is meant that the study has no available data, so we excluded the studies.

Q4: The authors note that the dietary assessment flavonoids intake varied across studies, with most

studies measuring intake using food frequency questionnaires and dietary history. In order for the readers to get a better feel of what the relative risks estimates (and their confidence intervals) from the 10 studies used in the meta-analysis mean, it would be helpful to mention how flavonoids intake was measured in each of the 10 studies. For example was intake measured in mg/day or mg per week, etc.?
R4: Thank you for your careful review. Flavonoids intake was measured in mg/day.

Q5: Table 2 (Stratified analyses of flavonoids intake and stroke risk) – the first group analysis titled "Overall studies" suggests that there were 10 studies categorized as Fatal/nonfatal stroke and 3 studies categorized as Ischemic stroke. However, the flow chart on page 15 suggests that 10 studies were included in the meta-analysis. Does this imply that the 'Ischemic stroke' group is a subset of the 'Fatal/nonfatal stroke' group?
R5: We are very sorry that we did not explain this critical point clearly. As described in the statistical analysis sections, when the result on total stroke in present meta-analysis was not available, we used data from ischemic stroke, nonfatal stroke, or fatal stroke as an equivalent to total stroke. As summarized in supplemental table 1, 10 studies included fatal/nonfatal stroke, 'ischemic stroke' group is not a subset of the 'Fatal/nonfatal stroke' group in this text.

Q6: Figures 3 (dose response relationship between dietary flavonoids intake and stroke risk) and 4 (Forest plot of flavonoids and risk of stroke) are hardly discussed in the text.
a. What do the different lines in figure 3 represent and what should the reader learn from the figure?
b. Only 4 out of the 10 studies were eligible for the dose-response analysis. What were the criteria for eligibility?
R6: Thank you for your constructive suggestion.
A. In figure 3, dose-response relationship between dietary flavonoids 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.
B. As described in the statistical analysis sections, in the dose-response analysis, the generalized least square for trend estimation method described by Greenland and Longnecker and Orsini et al was used to calculate study-specific slopes (linear trends) and 95% confidence intervals. The method requires the distributions of cases and person years for exposure categories, and 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.
We have made correction accordingly in the revised manuscript, and this amendment is highlighted in red.

Q7: Page 7: Limitation 1: I suggest you change the sentence to read "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 association between flavonoids intake and stroke risk". Thus these findings should be treated with caution. Page 8: Limitation 4 states that "we could not study the main sources of flavonoids because of the insufficient data". The authors should clarify what they mean by this.
R7: Thank you for your careful review. We have made correction accordingly in the strengths and limitations sections, and this amendment is highlighted in red.

Q8: The following sentences have typos/grammatical errors which should be corrected.
1. Page 4: Assessment of study quality. The last sentence that reads "Studies were graded as the high-quality if they met >8 awarded stars.

2. Page 4: Fourth sentence from the bottom - “All available data were conducted in the primary analysis”.
3. Page 5: Third sentence from top – separate the words ‘above’ and ‘mentioned’.
4. Page 6: Third sentence from top – the upper confidence limit should be 1 rather than 100.
5. Page 7: Strengths and limitations. The sentence that begins with ‘We’.
6. Page 12: Table 1, column 4 - the word range is misspelt.
R8: Thank you for your careful review. We have made correction accordingly in the revised manuscript, and this amendment is highlighted in red.

VERSION 2 – REVIEW

REVIEWER	Julia Peterson Tufts University, USA
REVIEW RETURNED	14-Dec-2015

GENERAL COMMENTS	Major problem (not specifically addressed in attached file) It appears that the dose-response is based on total flavonoids. Since the number and specific classes vary for each study, "total flavonoids" is not suitable for a dose-response analysis because the chemistry of each class is different. It would be better to do a dose-response analysis separately on each class (flavan-3-ols, flavanones, flavonols) that you have enough data. (You could probably include flavones with flavonols.) Minor changes are in the attached file which was originally an excel file, then a word file but attached as pdf and html. If anything is missing or unclear, I can send the editor the original file. The reviewer also provided a file with additional comments. Please contact the publisher for full details.

REVIEWER	Arno Greyling Unilever Research & Development Vlaardingen, The Netherlands AG is employed by Unilever R&D Vlaardingen. Unilever produces foods of which some are marketed to fit in a healthy diet and lifestyle.
REVIEW RETURNED	15-Dec-2015

GENERAL COMMENTS	The Authors have made some useful additions to improve the quality of their manuscript. Overall the piece is well written and succinct. Although I’m still unsure of the appropriateness of using flavonoid sub-classes as proxies of total flavonoid intake, the Authors clearly highlight this limitation, allowing the reader to make up her/his own mind with regard to the validity of the dose-response analysis. A few additional comments to consider: 1) The 2012 study by McCullough et al (Am J Clin Nutr. 2012 Feb;95(2):454-64. doi: 10.3945/ajcn.111.016634) also investigated the association between flavonoid intake and stroke risk in a large US cohort. I might have missed it, but it is not clear to me why this study did not fit the inclusion criteria of the current meta-analysis. Could the Authors elaborate? 2) Minor comments: - The portion of the discussion summarising

	potential mechanisms which may be responsible for the reduction in stroke risk (line 45 onwards) could be more succinct and perhaps focussed on more logical risk factors for stroke e.g. blood pressure (the addition on insulin resistance does not make sense to me). Additionally, the Author's reason for highlighting flavonols & isoflavones should be better explained. 3) Minor comments: - The legend of Figure 4 should contain more detail to make it clear that it relates to the dose-response analysis; Typo in Supplemental table 2 – "Adjusted covariates" in the top row should be "Flavonoid subclasses"?; Line 17 & 26, "flavonoids intake" and "flavonoids exposure" should be "flavonoid intake" and "flavonoid exposure"

REVIEWER	Bernet Kato New England Research Institutes (Massachusetts), USA
REVIEW RETURNED	31-Dec-2015

GENERAL COMMENTS	The authors have made the required revisions based on my comments

VERSION 2 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name
Julia Peterson
Institution and Country
Tufts University
USA

Q1: It appears that the dose-response is based on total flavonoids. Since the number and specific classes vary for each study, "total flavonoids" is not suitable for a dose-response analysis because the chemistry of each class is different. It would be better to do a dose-response analysis separately on each class (flavan-3-ols, flavanones, flavonols) that you have enough data. (You could probably include flavones with flavonols.)

R1: Once again, thank you for your careful review. We tried to do dose-response analyses separately on each class (such as flavanones, flavonels, flavan-3-ols, and isoflavones). But, we failed because of insufficient data (only two studies). Different sub-classes for flavonoid were used in the primary studies included in this meta-analysis. Among 11 studies, only 4 studies were eligible for the dose-response analysis, and the compounds of flavonoid subclasses are different among these studies, as summarized in Table 1. Of these, 2 studies included flavanones, 2 studies included flavones, 2 studies included flavonols, 2 studies included flavan-3-ols, and 2 studies included isoflavones. Due to the limited number of studies that met 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 sub-classes. As described in "Strengths and limitations", 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. Thus, these results should be treated with caution. Accordingly, we have addressed this important point in red in the revised manuscript

Table 1. Flavonoid subclasses and compounds for each study including dose-response meta-analysis.

Reference	Subclasses
Arts et al.9 2001	Catechins (belong to the flavonoid family)

Mink et al.14 2007
Flavanones, flavones, flavonols, isoflavones, flavan-3-ols or monomers, proanthocyanidins
Cassidy et al.16 2012
Flavanones, flavones, flavonols, flavan-3-ols, anthocyanins, polymers
Talaei et al.18 2014 Soy isoflavones
Abbreviation: Subclasses, which have available data on the dose-response analysis.

Q2: Minor changes are in the attached file which was originally an excel file, then a word file but attached as pdf and html. If anything is missing or unclear, I can send the editor the original file.
R2: We are very sorry that we did not explain those critical points clearly. We updated the search strategies and performed a more systematic search (updated on January 14, 2016) according to the comments. Finally, 11 studies met the inclusion criteria and were included in the meta-analysis. Accordingly, we have addressed those important points in red in the revised manuscript.

Reviewer: 2
Reviewer Name
Arno Greyling
Institution and Country
Unilever R&D Vlaardingen,
The Netherlands
Q1: The 2012 study by McCullough et al (Am J Clin Nutr. 2012 Feb;95(2):454-64. doi: 10.3945/ajcn.111.016634) also investigated the association between flavonoid intake and stroke risk in a large US cohort. I might have missed it, but it is not clear to me why this study did not fit the inclusion criteria of the current meta-analysis. Could the Authors elaborate?
R1: We are very sorry that we did not include the study by McCullough et al (Am J Clin Nutr. 2012 Feb;95(2):454-64. doi: 10.3945/ajcn.111.016634). We updated the search strategies and performed a more systematic search according to the comments. According to the inclusion criteria, this study should be selected for the current meta-analysis. We re-investigated the association between flavonoid intake and stroke risk after added the study by McCullough et al, this result was consistent with pre-added. Additionally, the 2012 study by McCullough et al was not included in dose-response analysis because of the lack of available data (such as person years for exposure categories). Accordingly, we have addressed this important point in the revised manuscript, and this amendment is highlighted in red.

Minor points:
Q1: The portion of the discussion summarising potential mechanisms which may be responsible for the reduction in stroke risk (line 45 onwards) could be more succinct and perhaps focussed on more logical risk factors for stroke e.g. blood pressure (the addition on insulin resistance does not make sense to me). Additionally, the Author's reason for highlighting flavonols & isoflavones should be better explained.
R1: Thank you for your careful review. Epidemiologic data suggest that dietary flavonols and isoflavones may reduce stroke risk and baseline measures of several stroke risk factors, including systolic and diastolic blood pressures, total cholesterol, and non-HDL cholesterol (Wang ZM, Zhao D, Nie ZL, et al. Flavonol intake and stroke risk: a meta-analysis of cohort studies. Nutrition 2014;30:518-23 and Sagara M, Kanda T, NJelekera M, et al. Effects of dietary intake of soy protein and isoflavones on cardiovascular disease risk factors in high risk, middle-aged men in Scotland. J Am Coll Nutr 2004;23:85-91). Corrections have been made accordingly in the revised version, and this amendment is highlighted in red.

Q2: The legend of Figure 4 should contain more detail to make it clear that it relates to the dose-response analysis; Typo in Supplemental table 2 – "Adjusted covariates" in the top row should be "Flavonoid subclasses"?; Line 17 & 26, "flavonoids intake" and "flavonoids exposure" should be

"flavonoid intake" and "flavonoid exposure"
R2: Thank you for your careful review and very constructive suggestion. We have made corrections accordingly in the revised manuscript, and all amendments are highlighted in red.

Reviewer: 4
Reviewer Name
Bernet Kato
Institution and Country
New England Research Institutes (Massachusetts), United States of America

Thank you for your careful review.

VERSION 3 - REVIEW

REVIEWER	Julia Peterson Tufts University School of Nutrition Science and Policy
REVIEW RETURNED	25-Feb-2016

GENERAL COMMENTS	Thank you so much for the MSWord file which is attached with suggested changes and comments. (I may have to make it a pdf.) A table of suggested changes for the figures and tables is inserted on page 11 at the end of the citations. On the last page of the attached file I have inserted a figure of some flavonoid classes. They vary at the C ring. My major problem with the paper is the dose response analysis. It is not the amount of flavonoid intake but the chemical structures of the flavonoids that is important. You must do dose response on the same flavonoid classes. According to your current supplemental table 2 and including the McCullough paper, for flavonols and flavones there are 9 studies, for flavanones 5 studies, for flavan-3-ols 3 studies, for anthocyanins 3 studies, for isoflavones 3 studies, and for proanthocyanidins 2 studies. Of course not all the studies have usable data. You could do a combined dose response analysis for the 5 studies that have flavonols, flavones, and flavanones. You cannot include Talaei because it only has isoflavones. I am hoping you really meant McCullough reference #17 and not Talaei reference #18 in the text as well as supplemental table 2. The reviewer also provided a marked copy with additional comments. Please contact the publisher for full details.

REVIEWER	Arno Greyling Unilever Reasearch & Development Vlaardingen AG is employed by Unilever Reasearch & Development Vlaardingen
REVIEW RETURNED	29-Feb-2016

GENERAL COMMENTS	Previous comments seem to be adequately addressed. I am still not convinced of the appropriateness of using flavonoid sub-classes as proxies of total flavonoid intake, however this limitation is clearly highlighted in the discussion. One minor comment - the supplemental tables do not seem to include any information on the study by McCullough et al - please add.

VERSION 3 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name
Julia Peterson
Institution and Country
Tufts University
USA
Q1: Thank you so much for the MSWord file which is attached with suggested changes and comments. (I may have to make it a pdf.) A table of suggested changes for the figures and tables is inserted on page 11 at the end of the citations. On the last page of the attached file I have inserted a figure of some flavonoid classes. They vary at the C ring.
R1: Thank you for your careful review and very constructive suggestion. We have made corrections accordingly in the revised manuscript and figures 2, 3 and 4 and Suppl tables 1 and 2, and all amendments are highlighted in red.

Q2: My major problem with the paper is the dose response analysis. It is not the amount of flavonoid intake but the chemical structures of the flavonoids that is important.
You must do dose response on the same flavonoid classes. According to your current supplemental table 2 and including the McCullough paper, for flavonols and flavones there are 9 studies, for flavanones 5 studies, for flavan-3-ols 3 studies, for anthocyanins 3 studies, for isoflavones 3 studies, and for proanthocyanidins 2 studies. Of course not all the studies have usable data. You could do a combined dose response analysis for the 5 studies that have flavonols, flavones, and flavanones. You cannot include Talaei because it only has isoflavones. I am hoping you really meant McCullough reference #17 and not Talaei reference #18 in the text as well as supplemental table 2.
R2: We are very sorry that we did not explain those critical points clearly. Once again, thank you for your careful review. We tried to do dose-response analyses separately on each class (such as flavonols, flavones, and flavanones). But, we failed because of insufficient data (only two studies). Different sub-classes for flavonoid were used in the primary studies included in this meta-analysis. Among 11 studies, only 3 studies were eligible for the dose-response analysis, and the compounds of flavonoid subclasses are different among these studies, as summarized in Table 1. Due to the limited number of studies that met 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 sub-classes. On the other hand, as described in “Statistical Analysis”, the dose-response analysis requires the distributions of cases and person years for exposure categories, and median/mean of flavonoid intake level for each comparison group. The McCullough et al (reference #17) did not reported person years for exposure categories, so we excluded in this dose-response analysis. For the reference #18, we admit that the chemical structures of the flavonoid are heterogeneous. Similarly, we used data from flavonols, flavones, flavanones, flavan-3-ols, and isoflavones (in the sequential order) as an equivalent to total flavonoid. Thus, we still included the article in this dose-response analysis. Finally, these results from the present dose-response analysis should be treated with caution.
Accordingly, we have addressed this important point in red in the revised manuscript

Reviewer: 2
Reviewer Name
Arno Greyling
Institution and Country
Unilever R&D Vlaardingen,
The Netherlands
Q1: Previous comments seem to be adequately addressed. I am still not convinced of the appropriateness of using flavonoid sub-classes as proxies of total flavonoid intake, however this limitation is clearly highlighted in the discussion.

One minor comment - the supplemental tables do not seem to include any information on the study by McCullough et al - please add.
R1: Thank you for your careful review. Accordingly, we have addressed this important point in the supplemental tables, and this amendment is highlighted in red.

VERSION 4 – REVIEW

REVIEWER	Julia Peterson Tufts University Friedman School of Nutrition Science & Policy, USA
REVIEW RETURNED	18-Apr-2016

GENERAL COMMENTS	If you want to do the dose response analysis on "total flavonoids", all the studies included should have at least 5 classes of flavonoid compounds. (I would not include the proanthocyanidins (or thearubigins) in the "total flavonoids" unless that class is present in all the articles. Other suggested changes are in the attached file. If the track changes and comments are stripped in converting the MSword doc to a pdf, tell the editor and I will send the file via email. Thank you for providing the source doc. The reviewer also provided a marked copy with additional comments. Please contact the publisher for full details.

VERSION 4 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name
Julia Peterson
Institution and Country
Tufts University
USA
Q1: If you want to do the dose response analysis on "total flavonoids", all the studies included should have at least 5 classes of flavonoid compounds. (I would not include the proanthocyanidins (or thearubigins) in the "total flavonoids" unless that class is present in all the articles.
Other suggested changes are in the attached file. If the track changes and comments are stripped in converting the MSword doc to a pdf, tell the editor and I will send the file via email. Thank you for providing the source doc.

R1: We are very sorry that we did not explain those critical points clearly. Once again, thank you for your careful review. We tried to do dose-response analyses using articles that have intakes of at least 5 classes. Among 11 studies, only 3 studies were eligible for the dose-response analysis, and the compounds of flavonoid subclasses are different among these studies, as summarized in Table 1. Due to the limited number of studies that met 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 sub-classes. On the other hand, as described in “Statistical Analysis”, the dose-response analysis requires the distributions of cases and person years for exposure categories, and median/mean of flavonoid intake level for each comparison group. The McCullough et al (reference #17) and Mursu et al (reference #15) did not reported person years for exposure categories, so we excluded in this dose-response analysis. For the reference #9, we admit that the chemical structures of the flavonoid are heterogeneous, such as catechin, flavan-3-ols, and so on. But, we still included the article in this dose-response analysis. As mentioned in Discussion, these results from the present dose-response

analysis should be treated with caution.
Accordingly, we have made corrections accordingly in the revised manuscript and Suppl tables 1 and 2, and all amendments are highlighted in red. Please see the latest revised manuscript.

VERSION 5 - REVIEW

REVIEWER	Julia Peterson Tufts University, USA
REVIEW RETURNED	12-May-2016

GENERAL COMMENTS	Thank you for the Word file. I hope the suggested changes and comments are not stripped when I upload the files. If they disappear, ask the editor to email me for the file directly. The reviewer also provided a marked copy with additional comments. Please contact the publisher for full details.

VERSION 5 – AUTHOR RESPONSE

Reviewer: 1
Reviewer Name
Julia Peterson
Institution and Country
Tufts University
USA
Q1: Thank you for the Word file. I hope the suggested changes and comments are not stripped when I upload the files. If they disappear, ask the editor to email me for the file directly.
R1: Thank you for your careful review. We have made corrections accordingly in the revised manuscript, and all amendments are highlighted in red.