Author's response to reviews

Title: Cruciferous vegetable intake is inversely associated with lung cancer risk among smokers: a case-control study

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Author's response to reviews: see over
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Sabina Alam, Ph.D.
Senior Scientific Editor
BMC Cancer

Dear Dr. Alam,

On behalf of all co-authors, I would like to thank you and the reviewers for their constructive comments. The manuscript has been extensively revised in response to these comments. Our responses are listed below:

**Reviewer 1 (Jennifer Lin)**

**Point 1.** “….the authors need to tone down when making inference. Suggest also removing the phrase of “strongly associated” when describing their findings”

**Response:** Modified as suggested.

**Point 2.** “It is not clear why unconditional rather than conditional logistic regression was used for the analysis given that cases were matched with controls”

**Response:** In our study, cases and controls were frequency-matched with an attempted control-to-case ratio of 2:1. In certain strata, fewer than 2 controls per case were available because of stringent matching criteria including age, gender, smoking status, and decade in which they completed the questionnaire. Therefore, the study is frequency-matched instead of exact matched, for which unconditional logistic regression model is more appropriate. The paper by Rothman and Greenland discussed this issue in detail, which is included as reference (24).

**Point 3.** “Discrepancy between findings needs to be addressed. The authors indicated that intake of cruciferous vegetable may be more relevant to smokers given the risk reduction present in smoking-induced lung cancer subtypes. However, intakes of vegetables and cruciferous vegetable were also inversely, albeit not significantly, associated with lung cancer in never smokers”

**Response:** We do not feel that there is discrepancy between findings. Our statement that intake of cruciferous vegetable may be more relevant to smokers is based on the findings obtained from stratified results (Table 3) showing that the inverse associations between both raw and total cruciferous vegetable intake and lung cancer risk were statistically significant among smokers, but not among never smokers. This statement is further supported by the results obtained in the lung cancer subtypes that are more strongly related to smoking (Table 5).
Point 4. “Need to perform interaction tests of smoking status, number of cigarettes, and smoking years…..provide p values in the text”

Response: Modified as suggested. The following sentences were added in the manuscript in the Results section (page 11, first paragraph): We also explored interactions between intake of each food category and smoking behavior in relation to lung cancer risk. No significant interactions were observed, except for total vegetable intake and years of smoking (p=0.0221). As shown in stratified results (Table 4), the inverse association between vegetable intake and lung cancer risk was statistically significant in those with 30 years or less of smoking, but not among the long-term smokers (> 30 years of smoking). The table of interaction analysis was attached as reference.

<table>
<thead>
<tr>
<th>Interaction</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vegetable / smoking status</td>
<td>0.7156</td>
</tr>
<tr>
<td>Vegetable / years of smoking</td>
<td>0.0221</td>
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<tr>
<td>Vegetable / cigarettes per day</td>
<td>0.1310</td>
</tr>
<tr>
<td>Fruit / smoking status</td>
<td>0.7268</td>
</tr>
<tr>
<td>Fruit / years of smoking</td>
<td>0.2826</td>
</tr>
<tr>
<td>Fruit / cigarettes per day</td>
<td>0.7294</td>
</tr>
<tr>
<td>Cruciferous vegetable / smoking status</td>
<td>0.9478</td>
</tr>
<tr>
<td>Cruciferous vegetable / years of smoking</td>
<td>0.1802</td>
</tr>
<tr>
<td>Cruciferous vegetable / cigarettes per day</td>
<td>0.5159</td>
</tr>
<tr>
<td>Raw cruciferous vegetable / smoking status</td>
<td>0.5804</td>
</tr>
<tr>
<td>Raw cruciferous vegetable / years of smoking</td>
<td>0.1141</td>
</tr>
<tr>
<td>Raw cruciferous vegetable / cigarettes per day</td>
<td>0.6236</td>
</tr>
</tbody>
</table>

Point 5. “Information on FFQ validation is not provided”

Response: The questionnaire was not directly validated, but is similar in structure to other validated instruments. Foods rarely eaten or with distinct flavor as cruciferous vegetables, are typically recalled validly. Using a similar brief 61-item FFQ to measure food intake, validity of recall of broccoli intake was between 0.49 and 0.69 (Salvini et al., 1989).

Point 6. “Were controls free of other cancers?”

Response: Yes, the controls were free of cancer. Control participants came to Roswell Park Cancer Institute with a suspicion of neoplastic disease, but were diagnosed with conditions other than cancer. This was addressed in Methods section (page 5, last paragraph).

Point 7. “Needs some language corrections before being published”
Response: As suggested, the manuscript was reviewed and edited by senior authors (native-English speakers).

Reviewer 2 (Antonio Agudo)

Point 1. “….the effect of cruciferous would be reinforced (if confirmed) if these three variables were mutually adjusted. In order to avoid collinearity, maybe the amount of vegetables ‘other than cruciferous’ instead of total vegetables could be used in this model.”

Response: This is a very solid suggestion. Actually, we did consider mutually adjustment of intake of cruciferous vegetables, total vegetables other than crucifers, and fruit in the multivariate models. After mutual adjustment, intake of other vegetables as well as fruits were no long significantly associated with lung cancer risk, but association between intake of cruciferous vegetables and lung cancer risk remained statistically significant (the highest quintile versus the lowest quintile, OR and 95% CI were 0.79 (0.56-1.12) for total other vegetables with p for trend = 0.4873, 0.83 (0.61-1.14) for fruits with p for trend = 0.3183, 0.68 (0.48-0.94) for cruciferous vegetables with p for trend = 0.019, and 0.66 (0.48-0.89) for raw cruciferous vegetables with p for trend = 0.0104). We did not want to emphasize this finding, because these three variables (vegetables other than crucifers, fruits, and cruciferous vegetables) are significantly, but moderately, correlated with each other, with correlation coefficients ranged from 0.32 to 0.50. In Discussion section of the manuscript (page 11, last paragraph), we did mention that when cruciferous vegetable was excluded from total vegetables, the association between intake of total other vegetables and lung cancer risk was no longer statistically significant among former smokers, but the significant association remained among never smokers, which reinforces the effect of cruciferous vegetable among smokers.

Point 2. “….it would be relatively simple having an estimate of total glucosinolates. This would help to disentangle the effect of such compounds (as precursors of ITC and I3C) from other components of cruciferous…."

Response: We did not estimate total glucosinolate intake from food frequency questionnaire (FFQ) data for the following three reasons. Firstly, food composition data for glucosinolates are not available for some of cruciferous vegetables included in the current study, such as collard greens, turnip greens, and mustard greens. Significant associations with lung cancer risk were observed for each of these vegetables (results not shown). Secondly, it is known that substantial loss of glucosinolates occurs in cooked vegetables (ranging from 18.1% to 59.2%) (McNaughton and Marks, 2003). However, we have cooked versus raw intake information only for broccoli, cauliflower, and cabbage; no information is available for Brussels sprouts, kale, turnip green, collard green, and mustard green. We have concerns about the accuracy of total glucosinolate estimates for this reason. Thirdly, different endpoint products can be formed from
hydrolysis of glucosinolates, including potential chemopreventive agents isothiocyanates (ITCs) and indoles (I3C), as well as other non-beneficial compounds nitriles and thiocyanates (Fahey et al., 2001). Therefore, estimate of glucosinolate intake does not represent exposure of ITC and I3C.

**Point 3.** “Different crucifers differ according to the type of glucosinolates….This could be somehow mentioned in the discussion”

**Response:** Modified as suggested. The following sentences were added in the Discussion section (page 13, last paragraph): “It is noteworthy that in addition to isothiocyanate-precursor glucosinolates, cruciferous vegetables also contain other types of glucosinolates such as indole-precursor glucosinolates. The type and total content of glucosinolates differ substantially among different cruciferous vegetables as well as within same vegetables under different culture conditions (Kushad et al., 1999). It is possible that indoles and other phytochemicals and nutrients in cruciferous vegetable such as carotenoids, vitamin C, folic acid, selenium, may also play a role in chemoprevention of lung cancer, whether or not in combination with isothiocyanates.

**Point 4.** “In order to provide an idea of the validity of information, the estimated intake of cruciferous among controls should be compared with available estimates of these foods in the same population.”

**Response:** This is another valid point. We did compare our intake data with other studies. In the Discussion section of the manuscript (page 15, first paragraph), we stated that daily intake of cruciferous vegetables in our study (25.3 g/day, accounting for 13.8% of all vegetable intake) is comparable to other studies conducted in North America area (daily intake ranges from 16 to 40 g with 5.6 to 15.4% of total vegetable intake), suggesting the detected association might apply in general population. The values represented median daily intake of cruciferous vegetables among both cases and controls. According to reviewer’s suggestion, we recalculated daily intake values only among controls (26.7 g/day, accounting for 14% of all vegetable intake) and modified in the manuscript.