Author's response to reviews

Title: Macronutrients, vitamins and minerals intake and risk of esophageal squamous cell carcinoma: a case-control study in Iran

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Author’s response to reviews: see over
Response to Reviewer #2

(MS: 6714475951821561)

Title: Macronutrients, Vitamins and Minerals Intake and Risk of Esophageal Squamous Cell Carcinoma in a Population at Risk for Esophageal Cancer

Version: 2
Reviewer: András Keszei

Reviewer's report:

Thank you for the opportunity to review the revised manuscript by Jessri and colleagues. My comments were answered by the authors and the manuscript has improved in my view. I have one comment regarding the analysis done in the revised work. In the fully adjusted models the authors adjust for duration of smoking measured in years, as well as smoking intensity measured in pack-years (Statistical analysis 2. paragraph). Since the calculation of pack-years already entails smoking duration, it is not practical to adjust for these two inherently correlated variables. I would suggest using either pack-year only or separate variables for duration and intensity measured by cigarettes (packs) smoked per day.

- Thank you very much for your thoughtful comment.

As per suggestion of the reviewer, “duration of smoking measured in years” was removed from all fully adjusted models and we solely used “pack-year” in the analyses, which entails both the “duration and intensity” of smoking. All data were re-analyzed accordingly and odds ratios (ORs) and 95% confidence intervals (CI) values were revised in “Tables 2-4”. This has been noted in the “statistical analysis” section on page 7, lines 144-145 and footnotes of Tables 2-4.

In addition, the manuscript text was revised to include the revised ORs (95% CIs) in the “abstract” section on page 2 lines 39-44, as follows:

“After adjusting for potential confounders, the risk of ESCC increased significantly in the highest tertiles of saturated fat [OR: 2.88, 95% CI: 1.15-3.08], cholesterol [OR: 1.53, 95% CI: 1.41-4.13], discretionary calorie [OR: 1.51, 95% CI: 1.06-3.84], sodium [OR: 1.49, 95% CI: 1.12-2.89] and total fat intakes [OR: 1.48, 95% CI: 1.09-3.04]. In contrast, being in the highest tertile of carbohydrate, dietary fiber and (n-3) fatty acid intake reduced the ESCC risk by 78%, 71% and 68%, respectively. The most cancer-protective effect was observed for the combination of high folate and vitamin E intakes (OR: 0.02, 95% CI: 0.00-0.87; p<0.001).”
It was also corrected in the “results” section on:

Pages 8 and 9 lines 175-181:

“In the fully-adjusted model, those in the highest tertile of SFA intake had 2.88 times higher ESCC risk (95% CI: 1.15-3.08; p-trend=0.01), followed by those in the highest intake tertile of cholesterol (OR: 1.53, 95% CI: 1.41-4.13; p-trend<0.001), discretionary calorie (OR: 1.51, 95% CI: 1.06-3.84; p-trend=0.002) and total fat intake (OR: 1.48, 95% CI: 1.09-3.04; p-trend=0.005). On the other hand, being in the highest tertiles of carbohydrate, dietary fiber and (n-3) fatty acid reduced the risk of ESCC by 78%, 71% and 68%, respectively. In the preliminary age- and sex- adjusted analysis (original matching criteria), a positive association also emerged with an increased protein intake, which was not significant in the fully-adjusted model.”

Page 9 lines 186-191:

“In the fully-adjusted model, the most protective effects against ESCC risk were associated with higher intakes of folate (OR: 0.08, 95% CI: 0.02-0.90; p-trend <0.001) and vitamin E intakes (OR: 0.11, 95% CI: 0.03-0.74; p-trend<0.001), closely followed by selenium (OR: 0.15, 95% CI: 0.01-0.76; p-trend<0.001), vitamin B₆ (OR: 0.17, 95%CI: 0.05-0.91, p-trend=0.003) and riboflavin intakes (OR: 0.22, 95%CI: 0.07-0.86; p-trend=0.01) (Table 3). Being in the highest tertile of sodium intake residual was associated with 1.49 fold increase in the ESCC risk (p<0.001).”

And page 9 lines 194-196:

“After mutual adjustment for several potential confounders, the combination of high intakes of both chemicals was associated with a strong protective effect against ESCC risk (OR: 0.02, 95% CI: 0.00-0.87; p<0.001).”

“Discussion” section was also revised accordingly on:

Page 10 lines 202-203:

“Most importantly, being in the highest tertile of joint folate and vitamin E intake was associated with 98 % reduction in the ESCC risk.”

Page 10 lines 214-217:

“According to the Dietary Guidelines for Americans 2005 [1], 12-20% of total energy intake could be taken from discretionary calories, while in the present study more than 50% of total calories consumed were from discretionary calories, which is of concern since those in higher tertiles of discretionary calorie intake had about 1.5 times higher risk of ESCC.”
“High intake of dietary fiber in the present study decreased ESCC risk by about 70%.”

“High intake of vitamin C in this study was associated with more than 60% reduction in ESCC risk.”

“In the present study, higher sodium intake was associated with almost 50% increase in the ESCC risk.”

“Calcium intake from foods in this study was associated with about 50% reduction in ESCC risk.”

Level of interest: An article whose findings are important to those with closely related research interests.
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests

References:

Response to Reviewer # 3

(MS: 6714475951821561)

Title: Macronutrients, vitamins and minerals intake and risk of esophageal squamous cell carcinoma: a case-control study in Iran
Version: 4
Date: 15 September 2011
Reviewer: Meira Epplein

Reviewer's report
Major comments:

1. The presentation of the case-control difference in percent complied with international nutrient guidelines does not add to this paper. This is especially true in light of the authors' own admission that the use of a semi-quantitative FFQ “is well-recognized for its weakness in quantification of nutrient intakes” (p. 19). I would remove the figures, and merely add a line or two in the discussion section (or introduction section) to note the general non-compliance with these guidelines among this population. If the authors would like to draw attention to this issue, I suggest a separate paper that would include multivariate ORs (as performed in Tables 1 and 2).

- Thank you for your comment.

As per suggestion of the reviewer, “Figures 1 and 2” were removed from the manuscript and the related sections in “abstract”, “materials and methods”, “result”, “discussion” in addition to their references were also excluded accordingly. In line with reviewer’s comment, few statements were added to “discussion” section to note the high nutrient intake inadequacy among Iranian population, on page 12 lines 252-254, as follows:

“On the other hand, the marked cancer-protective effect we observed for high folate intakes could be partly attributed to the comparatively high rates of folate intake deficiency, as more than 90% of cases and 50% of controls in this study had intakes below the Recommended Dietary Allowances (RDA) (data not shown) [1].”

A general statement was also added to “discussion” section on page 14 lines 308-310, as follows:

“Previous studies in Iran have reported high rates of vitamin/mineral deficiencies among EC patients [2-4]; which is in line with previous research showing deficiency of zinc [5], calcium [6] and potassium [7] to be widespread among EC patients.”

2. Because, as the authors correctly note, the numbers are very small in individual cells in these analyses, n’s should be included in Tables 1 and 2, so that the readers can see the number of cases that contributed to the ORs for each tertile. I realize that these tables are already very
large, so I suggest that the means and standard deviations, and cut-off points as presented in the footnotes, be removed to supplemental materials.

- We agreed to the reviewer’s comment.

Sample size in each cell was added to Tables 2-4 and all means, standard deviations, cut-off points and related footnotes were removed from “Tables 2-4” to “additional files 1 and 2”. The “result” section of manuscript was also modified to reflect this change, on page 8 lines 170-171:

“The calorie-adjusted mean values for selected macronutrients and relative risk estimates of ESCC by tertiles of macronutrient intake residuals are presented in Table 2 and Additional file 1.”

Page 9 lines 182-185:

“The adjusted mean intakes of vitamin A, β-Carotene, vitamin D, vitamin E, α-Tocopherol, thiamin, riboflavin, vitamin B₆, folate, vitamin B₁₂, vitamin C, iron, calcium, phosphorus, methionine and selenium were significantly higher among controls compared to ESCC cases (p<0.05), while average adjusted sodium intake was significantly higher among cases compared to the controls ( p<0.001) (Additional file 2).”

And on page 9 lines 186-191:

“In the fully-adjusted model, the most protective effects against ESCC risk were associated with higher intakes of folate (OR: 0.08, 95% CI: 0.02-0.90; p-trend <0.001) and vitamin E intakes (OR: 0.11, 95% CI: 0.03-0.74; p-trend<0.001), closely followed by selenium (OR: 0.15, 95% CI: 0.01-0.76; p-trend<0.001), vitamin B₆ (OR: 0.17, 95%CI: 0.05-0.91, p-trend=0.003) and riboflavin intakes (OR: 0.22, 95%CI: 0.07-0.86; p-trend=0.01) (Table 3). Being in the highest tertile of sodium intake residual was associated with 1.49 fold increase in the ESCC risk (p<0.001).”

3. Even after re-reading the methods section, I am confused as to the selection and categorization of potential confounders. Specifically:

a) I do not see how this study has the power to adjust for NSAIDs use, as none of the controls (as presented in Table 1) were identified as NSAIDs users.

b) Education level was categorized as <5 vs. #5 years, and yet only 4 controls, and no cases, reported #5 years of education (see Table 1). It appears that a more reasonable categorization would be illiterate vs. #1 year of education.

c) While the limitation of categorizing a continuous variable, such as age, is mentioned in the discussion section, it is still not explained why this variable was categorized. I would prefer to see it modeled as a continuous variable, unless there is a reasonable explanation of why that is not a good idea.

- Thank you for your thoughtful comments and apologies for this mistake.
a) As per suggestion of the reviewer, NSAIDs use was removed from the fully-adjusted models presented in Tables 2-4, due to its lack of use by controls. This was noted in all sections of the manuscript including, “abstract”, “statistical analyses”, “result”, “discussion”, and footnotes of tables 2-4. In addition, manuscript text was revised to include the revised Odds ratios (ORs) and 95% confidence intervals (95% CIs) in the “abstract”, “result” and “discussion” sections, accordingly (as detailed below).

b) Education level presented in Table 1, was re-categorized into two groups of “illiterate” and “literate”, to account for the small number of individuals in higher education categories. This was noted in footnotes of Table 2-4. In addition, education categories were also corrected in the “statistical analyses” section on page 7 line 145:

“The fully-adjusted model, on the other hand, included the following covariates: age (years), sex (male/female), GERD symptoms (yes/no), BMI (kg/m$^2$), smoking status (never/former/current), smoking intensity and duration (<20, ≥20 pack-years), physical activity (MET) (light/heavy), and education level (illiterate, literate).”

All regression models were re-generated to include the modified education categories and results were revised in the “abstract”, “results” and “discussion” sections as detailed below.

c) In line with reviewer’s comment and to avoid residual confounding, age was entered into models as a continuous variable. This change has been indicated in footnotes of tables 2-4, and in “statistical analysis” section on page 7 lines 141-146, as follows:

“The base model was adjusted for the matching variables, i.e. age (years) and sex (male/female), which are controlled for automatically by design. The fully-adjusted model, on the other hand, included the following covariates: age (years), sex (male/female), GERD symptoms (yes/no), BMI (≤24.9, >24.9 kg/m$^2$), smoking status (never/former/current), smoking intensity and duration (<20, ≥20 pack-years), physical activity (MET) (light/heavy), and education level (illiterate, literate).”
Based on the reviewer’s comment, the manuscript text was revised to include the revised odds ratios (ORs) and 95% confidence intervals (95% CIs) in the “abstract” section on page 2 lines 39-44, as follows:

“After adjusting for potential confounders, the risk of ESCC increased significantly in the highest tertiles of saturated fat [OR: 2.88, 95% CI: 1.15-3.08], cholesterol [OR: 1.53, 95% CI: 1.41-4.13], discretionary calorie [OR: 1.51, 95% CI: 1.06-3.84], sodium [OR: 1.49, 95% CI: 1.12-2.89] and total fat intakes [OR: 1.48, 95% CI: 1.09-3.04]. In contrast, being in the highest tertile of carbohydrate, dietary fiber and (n-3) fatty acid intake reduced the ESCC risk by 78%, 71% and 68%, respectively. The most cancer-protective effect was observed for the combination of high folate and vitamin E intakes (OR: 0.02, 95% CI: 0.00-0.87; p<0.001).”

It was also corrected in the “results” section on:

Pages 8 and 9 lines 175-181:

“In the fully-adjusted model, those in the highest tertile of SFA intake had 2.88 times higher ESCC risk (95% CI: 1.15-3.08; p-trend=0.01), followed by those in the highest intake tertile of cholesterol (OR: 1.53, 95% CI: 1.41-4.13; p-trend=0.001), discretionary calorie (OR: 1.51, 95% CI: 1.06-3.84; p-trend=0.002) and total fat intake (OR: 1.48, 95% CI: 1.09-3.04; p-trend=0.005). On the other hand, being in the highest tertiles of carbohydrate, dietary fiber and (n-3) fatty acid reduced the risk of ESCC by 78%, 71% and 68%, respectively. In the preliminary age- and sex- adjusted analysis (original matching criteria), a positive association also emerged with an increased protein intake, which was not significant in the fully-adjusted model.”

Page 9 lines 186-191:

“In the fully-adjusted model, the most protective effects against ESCC risk were associated with higher intakes of folate (OR: 0.08, 95% CI: 0.02-0.90; p-trend <0.001) and vitamin E intakes (OR: 0.11, 95% CI: 0.03-0.74; p-trend<0.001), closely followed by selenium (OR: 0.15, 95% CI: 0.01-0.76; p-trend<0.001), vitamin B<sub>6</sub> (OR: 0.17, 95% CI: 0.05-0.91, p-trend=0.003) and riboflavin intakes (OR: 0.22, 95% CI: 0.07-0.86; p-trend=0.01) (Table 3). Being in the highest tertile of sodium intake residual was associated with 1.49 fold increase in the ESCC risk (p<0.001).”

And page 9 lines 194-196:

“After mutual adjustment for several potential confounders, the combination of high intakes of both chemicals was associated with a strong protective effect against ESCC risk (OR: 0.02, 95% CI: 0.00-0.87; p<0.001).”
“Discussion” section was also revised accordingly on:

Page 10 lines 202-203:

“Most importantly, being in the highest tertile of joint folate and vitamin E intake was associated with 98% reduction in the ESCC risk.”

Page 10 lines 214-217:

“According to the Dietary Guidelines for Americans 2005 [8], 12-20% of total energy intake could be taken from discretionary calories, while in the present study more than 50% of total calories consumed were from discretionary calories, which is of concern since those in higher tertiles of discretionary calorie intake had about 1.5 times higher risk of ESCC.”

Page 11 line 229:

“High intake of dietary fiber in the present study decreased ESCC risk by about 70%.”

Page 14 lines 297-298:

“High intake of vitamin C in this study was associated with more than 60% reduction in ESCC risk.”

Page 14 line 302:

“In the present study, higher sodium intake was associated with almost 50% increase in the ESCC risk.”

And page 14 lines 310-311:

“Calcium intake from foods in this study was associated with about 50% reduction in ESCC risk.”

4. The discussion section includes too many explanations of biological mechanisms that are not specifically pertinent to the present study. Most of these descriptions are of the general potential carcinogenic (or anti-carcinogenic) effect of various nutrients. More salient would be the mechanisms that are specific to ESCC – listing possible mechanisms for incidence of any cancer does not add to the discussion, and contributes to the excessive length of this section.

- Thank you for your comment, we agreed to the reviewer’s comment.
All general statements describing potential carcinogenic (or anti-carcinogenic) effects of nutrients on “any cancers” were removed from “discussion” section and associated references were also excluded.

**Minor comments:**

1. In the methods section, p. 6, it is reported that previous studies have reported “good correlations between dietary intakes assessed by the FFQ and those obtained from 24-h dietary recalls.” Please report the relevant correlations here.

   - Thank you for your helpful comment.

   As per suggestion of the reviewer, correlation coefficients between dietary intakes assessed by the FFQ and those obtained from 24-hour dietary recalls were reported in the “materials and methods” section on pages 5 and 6 lines 113-120, as follows:

   “A comparison of crude, energy-adjusted and deattenuated correlation coefficients for overall nutrient intakes between 24-h dietary recalls and this FFQ have been 0.44 and 0.37 in ≤35 and >35 year-olds, respectively, and for individual nutrients it ranged from 0.24 to 0.71 in men and from 0.11 to 0.60 in women. On the other hand, the mean reliability coefficients, ranged from 0.48 in ≤35 year-olds to 0.65 in >35 year-olds. This FFQ produced exact agreement rates ranging from 39.6% to 68.3% in men and from 39.6% to 59.1% in women, respectively. The validity coefficients, with the sample correlation between the questionnaires and biological markers as the lower limit and the estimates from the triad method as the upper limit were 0.21-0.56 for protein and 0.37-0.61 for energy [9].”

2. The results section is much too wordy, primarily due to the repetition of the reporting of specific results already presented in the tables. I would remove specific numbers (such as average intakes as presented on p. 10) and instead state the fact (e.g., “Cases consumed significantly more SFA and discretionary calories compared to controls.”) and refer the reader to the appropriate table.

   - Thank you very much for this comment.

   In line with the reviewer’s comment, average intake values were removed from the “result” section and readers were referred to appropriate tables/additional files on page 8 lines 172-175:

   “Cases consumed significantly more SFA and discretionary calories (energy derived from solid fat and added sugar), compared to the controls (p=0.006). On the other hand, controls consumed significantly more (n-3) fatty acids, dietary fiber, carbohydrate and vegetable oil than their case peers (p=0.04).”
And on page 9 lines 183-187

“The adjusted mean intakes of vitamin A, β-Carotene, vitamin D, vitamin E, α-Tocopherol, thiamin, riboflavin, vitamin B₆, folate, vitamin B₁₂, vitamin C, iron, calcium, phosphorus, methionine and selenium were significantly higher among controls compared to ESCC cases (p<0.05), while average adjusted sodium intake was significantly higher among cases compared to the controls ( p<0.001) (Additional file 2). Controls consumed 623.5 times as much selenium (p<0.001), 5.48 times as much β-carotene and 1.98 times as much α-tocopherol as the amount ESCC cases consumed.”

3. The results of the joint effects of vitamin E and folate (Table 4) should be presented more clearly so to be easier for the reader to assess. Specifically:
   a) only report ORs and CIs to two decimal points
   b) show the sample size (n) for each cell
   c) present the P for interaction

   • Thank you for your thoughtful comment.

Based on the reviewer’s suggestion, Table 4 was modified to report ORs and 95% CIs to two decimal points and also to present the sample size in each cell. In addition, “P for interaction”, was calculated using logistic regression analysis and is presented in the footnote of Table 4.

These modifications were reflected in “abstract” section on page 2 lines 43-44:

“The most cancer-protective effect was observed for the combination of high folate and vitamin E intakes (OR: 0.02, 95%CI: 0.00-0.87; p<0.001).”

In addition, this is corrected in “result” section on page 9 lines 195-198:

“After mutual adjustment for several potential confounders, the combination of high intakes of both chemicals was associated with a strong protective effect against ESCC risk (OR: 0.02, 95% CI: 0.00-0.87; p<0.001).There was a statistically significant interaction between vitamin E and dietary folate intake when evaluated in the model (p-value for interaction=0.03).”

This was also corrected in “discussion” section on page 10 lines 203-204, as follows:

“Most importantly, being in the highest tertile of joint folate and vitamin E intake was associated with 98 % reduction in the ESCC risk.”

4. If it is thought that the effect of a high fat diet might be mediated by BMI (p.13), it would also be worthwhile to examine the possibility of effect modification by BMI.

   • Thank you very much for your thoughtful comment.
In line with the reviewer’s comment, potential effect modification by BMI was assessed.

Although ESCC risk was higher among those with higher BMI values (>24.9) compared to those with lower BMIs (≤24.9), p for interaction was not significant (p for interaction=0.68).

This was added to the “discussion” section on page 10 lines 218-221, as follows:

“Since all our analyses were adjusted for usual adult BMI, the risk-enhancing effect of high fat diet on ESCC observed in the present study was independent of adiposity, which is a strong risk factor for carcinogenesis. Further effect modification by BMI revealed that although ESCC risk was higher among those with higher BMI values, p for interaction was not significant (data not shown).”

5. It is noted in the discussion (p. 14) that the association of a protective effect of high consumption of carbohydrates might be due to its association with lower intake of fat and/or high intake of fruits and vegetables. To add to this discussion, these variables should be put in the same model and the results reported, or at the least the actual correlations between these variables in this population should be reported.

- We agreed to the reviewer’s comment.

Correlation coefficient between carbohydrate intakes and fat intake was previously reported in “discussion” section. However, in agreement with reviewer’s suggestion, the correlation coefficient between “fruits and vegetable” intakes and “carbohydrate” intake was also calculated and reported in “discussion” section on page 11 lines 235-239, as follows:

“However, carbohydrate intake was also negatively correlated with fat intakes (correlation coefficient= -0.615) and hence a higher percentage of carbohydrate may just reflect lower intakes of fat and explain its inverse association with ESCC [10]. In addition, higher consumption of carbohydrate could be reflection of more plant-based food intakes, and especially fruit and vegetable; although in the present research, the correlation between carbohydrate and fruits and vegetable intakes was not significant (r=0.064).”

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests: I declare that I have no competing interests.
References:


