Author's response to reviews

Title: Use of vitamin supplements and risk of total cancer and cardiovascular disease among the Japanese general population: A population-based survey

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Author's response to reviews: see over
Response to Referee #1:

Thank you very much for your kind comments and suggestions, which have enabled us to improve our manuscript. Responses to each point you raised are provided below.

- Major Compulsory Revisions

Comment 1: The major ask is for a rewrite of the whole paper with an update of recent and past non-cited publications. I have added at the end a small example of relevant papers not included after a quick survey of the literature.

Response 1: Thank you very much for your helpful advice and examples. We followed your advice and tried to quote all relevant references. However, we found over 100 references. Therefore, we have rewritten our manuscript with updated references, based on the following three points, because our study focused on the incidence of cancer and CVD in this prospective cohort study.

1: For intervention trials, included Cochrane database and the trials that we quoted in other parts of our paper.
2: Excluded the studies for risk of mortality only, except the studies we quoted in other parts.
3: Excluded the studies published before 2000, except the studies we quoted in other parts.

Please see Ref 3-10, 12, 15, 17, 21-23, 29, 33, 34 in the revised manuscript.

Background
Paragraph 1

Comment 2: As stated above, you need to update your references up to 2010 references and include relevant consensus statements and Cochrane reviews (as per references 2, 4, 6, 11, 17, 18).

Response 2: We have updated references based on your recommendation, as follows:
Despite the popular use of vitamin supplements, the strong consumer belief that they prevent chronic diseases such as cancer and cardiovascular disease (CVD) [Ref 1, 2 in the revised manuscript], and the results from randomized controlled trials are mixed [Ref 3-10 in the revised manuscript]. Most randomized controlled trials show little support of a preventive effect of vitamin supplement use and even increased risk [Ref 6,7 in the revised manuscript] for cancer and CVD incidence and mortality, with some exceptions [Ref 8-10 in the revised manuscript]. However, data of randomized controlled trial suffer from concerns about overreliance on secondary rather than primary prevention, insufficient intervention and follow-up periods, particularly regarding the incidence of cancer, inappropriate supplement doses, and unsuitable cohorts for testing the hypothesis. Therefore, studies for the effects of long-term, low doses of several agents in the general population are needed. Despite several prospective cohort studies investigating their effect on cancer incidence (all site [Ref 11, 12 in the revised manuscript], colorectal [Ref 13-15 in the revised manuscript], breast [Ref 16, 17 in the revised manuscript], lung [Ref 18, 19 in the revised manuscript], prostate [Ref 20-23 in the revised manuscript], non-Hodgkin lymphoma [Ref 24 in the revised manuscript]), and CVD incidence [Ref 11, 25-29 in the revised manuscript], scientific data supporting their benefits remain controversial. (Page 4, line 2-14)

Paragraph 2
Comment 3:  You state that there are no data for a prospective cohort study in Asia. Maybe, but there has been an intervention trial in China in 1996 – this needs to be stated (references 11, 12). There are also other intervention trials (references 8, 9, 19).

Response 3:  Thank you very much for your comments regarding the intervention trial in Asia. We believe that it is very important that risk for cancer and cardiovascular disease are assessed in a large prospective cohort study, as well as in an intervention trial. It is because that data of intervention trial suffer from concerns about overreliance on secondary rather than primary prevention, insufficient intervention and follow-up periods, particularly regarding the incidence of cancer, inappropriate supplement doses, and unsuitable cohorts for testing the hypothesis. Therefore, studies for the effects of long-term, low doses
of several agents in a general population are needed. We have included the above points with uploaded references of intervention trials in the Background section, as follows:

However, all prospective studies have been conducted in Western populations (United States [Ref 11-15, 17-29, 31-34 in the revised manuscript] and European countries [Ref 16, 19, 25 in the revised manuscript]). No data have been reported for prospective cohort studies in Asian general populations, although there are some randomized clinical trials [Ref 8, 40 in the revised manuscript]. (Page 4, line 27-31)

Methods

Paragraph 1

Comment 4: Need to talk about the characteristics of your 16% drop outs.

Response 4: We have reported the characteristics of non-respondents to a first survey in the part of our cohort [Ref 116 in the revised manuscript]. Risks of mortality for all causes, all cancers, and CVD were higher among non-respondents compared with respondents. Elevated risk for cancer was observed only in the first 2 years of follow-up, whereas that for stroke was relatively stable for the entire period. Therefore, the possibility of selection bias needs to be considered when generalizing the present findings, because 16% of the eligible subjects did not reply in the second survey. We have added this point in the Discussion section, as follows:

In addition, the possibility of selection bias needs to be considered when generalizing the present findings because 16% of the eligible subjects did not reply in the second survey. In our previous report, risks of mortality for all causes, all cancers, and stroke were higher among non-responders to the first survey compared with respondents and elevated risk for cancer was observed only in the first 2 years of follow-up, whereas that for stroke was relatively stable for the entire period [Ref 66 in the revised manuscript]. (Page 13, line 9-14)
Paragraph 3
Comment 5: Need to update your follow up – it is now 2011, some 5 years since the last update.

Response 5: We have a lag time to update the occurrence of cancer and cardiovascular disease in our registry system. This is the longest follow-up period to ensure a sufficient quality across all the public health center areas of the registries of cancer and cardiovascular disease.

Paragraph 5
Comment 6: Why did you not adjust for vitamin E, vitamin C, and vitamin D?

Response 6: As you pointed out, we further adjusted for dietary \( \alpha \)-tocopherol, vitamin C, and vitamin D intakes. Our finding remained unchanged when adjusted separately or simultaneously. Based on your suggestion, we have included this point in the Method and Results, as follows:

A residual model was used for energy adjustment of green vegetable consumption, vitamin B_2, vitamin B_6, vitamin B_{12}, folate, \( \alpha \)-tocopherol, vitamin C, and vitamin D intake reported in food-frequency questionnaire [Ref 55 in the revised manuscript]. (Page 8, lines 9-11)

These statistically significant findings remained unchanged when we further adjusted dietary vitamin B_2, B_6, B_{12}, folate, \( \alpha \)-tocopherol, vitamin C, and vitamin D intake separately and simultaneously (data not shown). (Page 10, lines 22-24)

Results
Paragraph 1
Comment 7: Give number as well as percentages.

Response 7: In line with your recommendation, we have added this information in the Result section, as follows:

Of the participants included in this analysis, 49,060 subjects (78.3%) reported
no vitamin supplement use, 7833 subjects (12.5%) reported only past vitamin supplement use (in the first survey), 2593 subjects (4.2%) reported only recent vitamin supplement use (in the second survey), and 3143 subjects (5.0%) reported past and recent vitamin supplement use. Among subjects who used vitamin supplements and reported the brand name in the second survey, the most common vitamin supplement was B vitamins for men and women (multivitamin: 474 subjects [25.8%] and 566 subjects [19.6%]; antioxidants: 30 subjects [1.6%] and 26 subjects [4.4%]; vitamin A: 65 subjects [3.5%] and 144 subjects [5.0%]; B vitamins: 797 subjects [43.5%] and 883 subjects [30.6%]; vitamin C: 299 subjects [16.3%] and 656 subjects [22.7%]; vitamin E: 295 subjects [16.1%] and 843 subjects [29.2%]; other vitamins: 219 subjects [11.9%] and 443 subjects [15.3%], respectively). (Page 8, line 17-27)

Paragraph 2
Comment 8: Please give p values in Table 1 and convert proportions in this paragraph to odds ratios with 95% confidence intervals as follow up to your statement of differences.

Response 8: Following your suggestion, we have compared among the four groups statistically. Odds ratios and 95% confidence intervals were not presented, but P values by analysis of variance or χ²-test were listed to statistically show the difference. Please see New Table 1. We also added this information in the Result section, as follows:

Individuals with past use and consistent use of vitamin supplements were significantly older for both sexes. Men who had never used supplements were thought to have lower health consciousness due to higher proportions with a BMI ≥25 kg/m², a greater likelihood of being a smoker or regular drinker, less information on their disease history (angina, diabetes, colonic polyp, and hepatitis), fewer screening examinations, and less consumption of soy foods and fruits compared with other men. Significantly higher proportions of men with consistent supplement use took more medications (hyperlipidemia and diabetes), were more likely to have disease histories (angina, diabetes, duodenal ulcer, colonic polyp, and hepatitis), and may have higher health consciousness suggested by lower BMI, less regular drinking, more screening
examinations, and higher consumption of fruits. Men with past supplement use also had a significantly higher proportion of antihypertensive medication use. Men with recent use also tended to have a healthy lifestyle and significantly lower proportions were smokers or taking diabetic medication. Women who had never used supplements were likely to have a healthier lifestyle, with significantly lower proportions being smokers or regular drinkers than other women. Women with recent or consistent use were also basically health conscious, having a lower BMI and a higher proportion of screening examinations, despite there being a significantly higher proportion of regular drinkers. Individuals with consistent use also consumed significantly larger amounts of fruits, folate, and vitamin C. They also tended to have significantly higher proportions of medication use (hypertension and hyperlipidemia) and history of diseases such as gastric and colonic polyps than those who never used supplements. Women with recent use were also more likely to have a history of gastric and colonic polyps, despite their younger age, and had a significantly higher proportion of medication use except for hypertension, hyperlipidemia, and diabetes. Women with past use tended to have an unhealthy lifestyle, including a higher BMI and a greater likelihood of smoking and medication use (hypertension and diabetes). (Page 8, line 30- Page 9, line 23)

Comment 9: Table 1, there was an error in number of ‘never use’.

Response 9: We have looked over the number of ‘never use’ in Table 1, but we could not find an error. Please see New Table 1. If we have misunderstood your comment, however, please let us know.

Paragraph 3
Comment 10: Table 2 and 3 I think have been mixed up. Need to be switched as they don’t correlate with text (males, females).

Response 10: Accordance with your suggestion, we combined up Table 2 and Table 3. Please see New Table 2.
Comment 11: At the end of this paragraph, you are reporting risks that are not significant as well as significant – need to clarify this.

Response 11: Following the comment from Referee #2, we have omitted this sentence in the revised manuscript. Please see Comment 9 from Referee #2.

Paragraph 4
Comment 12: Need to say whether these findings are significant or not.

Statistical significance of increased cancer risks for recent and past use and decreased cardiovascular disease risk for consistent use among women remained when we further adjusted dietary vitamin intakes. Following your suggestion, we also have added this information in the Result section, as follows:

These statistically significant findings remained unchanged when we further adjusted dietary vitamin B₂, B₆, B₁₂, folate, α-tocopherol, vitamin C, and vitamin D intake separately and simultaneously (data not shown). (Page 10, lines 22-24)

Comment 13: Why didn’t you adjust for vitamin C and E?

Response 13: As I mentioned in “Response 6”, we further adjusted for α-tocopherol, vitamin C, and vitamin D and found similar results. We have mentioned this in the Method and Results section. Please see Response 6.

Paragraph 5
Comment 14: You need to report significant interactions before you stratify; this is the statistical reason for stratification.

Response 14: We had found no significant interactions (all P values for interaction > 0.5). Following your suggestion, we added this information and omitted the results from stratified analyses in the Results section.
Age, smoking status, alcohol intake, and dietary intake of vitamin $B_2$, $B_6$, $B_{12}$, folate, $\alpha$-tocopherol, vitamin C, and vitamin D did not significantly interact with any of the above results (for all interactions, $P > 0.5$). 

**Discussion**

**Paragraph 2**

Comment 15: Need to update with relevant references and need to give details of relevant studies, as well as in the 2 you have given you need to give more details eg number of people in study and number of cases of CHD and cancer in each study.

Response 15: Thank you for your suggestion. We have updated with relevant references and found the only two references that clarify the consistency of vitamin supplement use over two surveys, which we had already shown in our manuscript. As you suggested, we have added the details of these studies, as follows:

One prospective cohort study in the United States investigated consistency for vitamin supplement use through two surveys among 145,260 subjects, observing 797 incident cases of colorectal cancer, and found that multivitamin supplement use in the first survey and in both surveys was associated with reduced risk of colorectal cancer, whereas multivitamin supplement use in the second survey had no association with the disease [Ref 13 in the revised manuscript]. Another study, in which 3490 deaths were observed among 11,178 study subjects in the United States, found that use of vitamin E supplements at two points within a relatively short period (baseline and study inception 3 years earlier) was associated with reduced risk of coronary heart disease mortality, whereas use at one point did not show significant association in multivariate analysis [Ref 31 in the revised manuscript].
Paragraph 3
Comment 16: Need to discuss ATBC results as this is really relevant to vitamin and cancer, and there has been huge debate in the literature. Also, if you think the results are due to selection bias, need to give more detail of this from your study.

Response 16: As you pointed out, the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) trial and the Beta-Carotene And Retinol Efficacy Trial (CARET) were the relevant intervention trials. We think there was some selection bias (high-risk groups, such as heavy smokers and history of exposure to asbestos) and using high supplement doses. Therefore, to generalize findings, it a prospective study would be needed as well as an intervention trial. As you suggested, we have discussed this point in the Discussion section, as follows:

Furthermore, high-dose antioxidant supplementation might cause an increased risk of cancer among a high-risk group; in addition, two large, randomized clinical trials in which high doses of β-carotene were used, the Beta-Carotene And Retinol Efficacy Trial (CARET) in the United States and the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) trial in Finland, found that β-carotene, alone or in combination with vitamin E or retinyl palmitate, increased the incidence of lung cancers compared with placebo among high-risk groups, such as heavy smokers and those with a history of exposure to asbestos [Ref 6, 7 in the revised manuscript]. (Page 12, line 19-26)

Paragraph 4
Comment 17: What do you mean by characteristics of the subjects, which ones?

Response 17: Women with past use tended to have an unhealthy lifestyle, including a higher BMI and a greater likelihood of smoking, and medication use (hypertension and diabetes). On the other hand, women with recent use were more likely to have a history of gastric and colonic polyps, despite their younger age, and had a significantly higher proportion of medication use except for hypertension, hyperlipidemia, and diabetes. Elevated risk may be partly
explained by characteristics of women, which were not measured or could not be controlled for in our study. We added this information in the Discussion section, as follows:

Women with past use tended to have unhealthy characteristics, such as a higher BMI and a greater likelihood of smoking, and medication use (hypertension and diabetes). Recent use in women may have been prompted by symptoms of ill health, because women with recent use had a higher proportion of disease histories despite their younger age, such as a history of gastric and colonic polyps, and had a significantly higher proportion of medication use except for hypertension, hyperlipidemia, and diabetes. Furthermore, the association of cancer with recent use was not significant when we estimated the HR after excluding women diagnosed as having cancer within 5 years of baseline, though that might be partly caused by the decreased number of cases. Elevated risk may be partly explained by characteristics of the women that were not measured or could not be controlled for in our study. (Page 12, line 7-16)

**Paragraph 5**

**Comment 18:** Need to talk about drop out rates

**Response 18:** Please see ‘Response 4’.

**Paragraph 6**

**Comment 19:** Not true. Losonczy, Harris & Havlik compared CVD and cancer in the same study (your reference 19)

**Response 19:** In our study, we had focused on the incidence of cancer, coronary heart disease, and stroke in the same study. Losonczy, et al. demonstrated the mortality (coronary heart disease and cancer). We have added this point in the Discussion section, as follows:

To our knowledge, this is the first prospective cohort study to examine associations between vitamin supplement use pattern and risk of cancer and
cardiovascular disease incidence simultaneously.  (Page 13, line 20-22)
A small selection of relevant references

Level of interest: An article of importance in its field

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:
'I declare that I have no competing interests'
Response to Referee #2:
Thank you very much for your kind comments and suggestions which have enabled us to improve our manuscript. The following are our responses to your comments.

Comments:
Comment 1. Methods, 1st para, line 9: We excluded 5,809 persons… This seems to be incorrect. 95,405-5,809 does not equal 95,327.

Response 1: Thank you for your letting know of this error. We have corrected the number of eligible and non-eligible subjects in the Method section, as follows:

Of 116,896 people in nine public health areas, 95,405 (82%) individuals responded to the first survey. We excluded 1168 persons who were not Japanese, who had died or moved out of a study area, or who were lost to follow-up before the starting point. This left 94,237 eligible subjects. In 1995 and 1998, the second survey was conducted; 79,809 subjects replied (84%; 36,783 men and 43,026 women) and were included in the present study. (Page 5, line 14-18)

Comment 2. Methods, 4th para, line 1: I am not familiar with the term “active patients’ notification”. Is this some sort of admission-to-hospital data?

Response 2: In some areas, major hospitals care for most of the patients with cancer or cardiovascular disease (up to 80%). Then local major hospitals are considered as the primary data source, and clinical information is extracted from medical records into cohort-specific registration forms either by physicians in the hospital or physicians in the public health center [Ref 44 in the revised manuscript]. We have defined these extractions as the term “active patients’ notification.” We added this information in the Method section, as follows:

The occurrence of cancer was identified by active patients’ notification from major local hospitals in the study area, that is, the extraction of clinical information from medical records into cohort-specific registration forms in
either local major hospitals, which care for most of the patients with cancer or CVD (up to 80%) in some areas, by physicians in the hospital or physicians in the public health center [Ref 44 in the revised manuscript], and from data linkage with population-based cancer registries, with permission from each of the local governments responsible for the cancer registries. (Page 6, line 16-22)

Comment 3. Methods, 4th para, line 4: “Cases of cancer were coded according to the WHO”. I suppose this means that the ICD was used, but what version?

Response 3: We coded cases of cancer according to the International Classification of Disease for Oncology, third edition [Ref 45 in the revised manuscript]. We have added this information in the Method section, as follows;

Cases of cancer were coded according to the *International Classification of Disease for Oncology*, third edition, of the World Health Organization [Ref 45 for cohort studies in the revised manuscript]. (Page 6, line 22-23)

Comment 4. Methods, last paragraph (rather long…), description of multivariate model: Is “screening examination” one or more variables? Does each examination correspond to one yes/no variable? How was this information collected?

Response 4: Information on “screening examination” was obtained by self-administered questionnaire asking yes/no for each examination (blood pressure measurement, biochemical examination, electrocardiogram, fundus examination, chest radiograph, sputum cytology, gastric photofluorography, gastrointestinal endoscopy, fecal occult blood test, barium enema, or colonoscopy for men and women, and mammography or Papanicolaou smear for women). If a subject replied with “yes” to at least one examination, we regarded the subject as taking examination. We have added this point in the Method section, as follows:

In the multivariate model, we further adjusted for smoking status, ……, current
medication status (hypertension, hyperlipidemia, or diabetes mellitus), and screening examination (blood pressure measurement, biochemical examination, electrocardiogram, fundus examination, chest radiograph, sputum cytology, gastric photofluorography, gastrointestinal endoscopy, fecal occult blood test, barium enema, or colonoscopy for men and women, and mammography or Papanicolaou smear for women), which were reported in a questionnaire in the second survey. As for current medication status and screening examination, if a subject replied with “yes” to at least one medication or examination, we regarded the subject as using medication or taking the examination, respectively. (Page 7, line 23- Page 8, line 6)

Comment 5. Results, 3rd para: I am not sure of what is meant by the term “ischemic infarction”. Is it an ischemic stroke/brain infarction, or a myocardial infarction? What ICD codes are included?

Response 5: We meant the term as an ischemic brain infarction. The occurrence of cardiovascular disease was not based on ICD codes, but on the criteria of the MONICA project for myocardial infarction and the National Survey of Stroke for stroke. We have revised the term in the Result and Discussion sections, as follows:

When we performed separate analyses for coronary heart disease, hemorrhagic stroke, or ischemic brain infarction, decreased risk was observed for ischemic brain infarction with statistical significance with consistent use (coronary heart disease: HR 0.19, 95% CI 0.03–1.34; hemorrhagic stroke: HR 0.61, 95% CI 0.29–1.31; ischemic brain infarction: HR 0.52, 95% CI 0.28–0.98). (Page 10, line 14-18)

In the present study, the inverse associations for CVD, especially for ischemic brain infarction, was observed with consistent supplement use in women. (Page 11, line 22-23)

Therefore, the inverse association between the consistent use of vitamin supplement and risk of CVD in women, especially ischemic brain infarction, might be caused by supplementation with B vitamins. (Page 12, line 1-4)
Comment 6. Discussion, 3rd para, 2nd sentence: “homocysteine may promote atherogenesis”. I am not an expert on cardiovascular pathogenesis by any means, but as far as I know there is very little evidence that lowering homocysteine in itself lowers cardiovascular risk. On the contrary, I believe there is fairly good evidence that it does not affect risk. References 39-41 are rather old (the most recent is from 1997). I strongly suggest an update here.

Response 6: As you mentioned, there is very little evidence that lowering homocysteine in itself lowers coronary heart disease risk. In contrast, some studies showed possible evidence for stroke [Ref 9, 10 in the revised manuscript]. Because stroke is more common than coronary heart disease in Japanese (Ueshima H, et al. J Atheroscler Thromb. 2007), the protective trend of vitamin B for cardiovascular diseases, especially for stroke, might be observed in our study. We have added more details for this point in the Discussion section and updated references, as follows;

It is known that homocysteine may promote atherogenesis by damaging the vascular matrix, increasing the proliferation of endothelial cells and facilitating oxidative injury to vascular walls [Ref 56-58 in the revised manuscript] and may be related to cardiovascular disease [Ref 59, 60 in the revised manuscript]. Although several large trials of homocysteine-lowering B-vitamin therapy have all failed to demonstrate a reduction in coronary heart disease risk, some studies have shown possible evidence for stroke [Ref 9, 10 in the revised manuscript].

Discretionary revisions:

Comment 7. Results: I suppose the authors want to avoid unnecessary numbers, but this writer is fond of standard errors of proportion. For example, I would have liked to see the SE(p):s for the prevalence of vitamin supplement types, although I realize this probably would have meant another table.

Response 7: We have added this information in the revised table. For age and metabolic equivalent task, we have changed into SE from SD. For food and dietary nutrient intakes, we have added SE.
Comment 8. Similarly, there are no statistical tests for the results in Table 1. Although not strictly necessary, this writer would like to have them. Maybe the material is so large that all differences are significant at least on a 5% level, but if so, I think it should be mentioned.

Response 8: Following your suggestion, we have added this information in the revised table and in the Method section. Please see New Table1 and ‘Response 8’ to Referee #1.

Comment 9. Results, 3rd para: The 4th sentence, starting with “Although not…”: I think this sentence should be omitted. This seems like possible mass significance, since it is a subgroup analysis of an overall null association. Same para, last sentence: A not very significant result, I suggest omission of this sentence.

Response 9: Following your recommendation, we have omitted these sentences and changed the 3rd sentence in the revised manuscript, as follows:

No significant association was found between any specific vitamin supplement use in the second survey and total cancer and CVD. [Page 9, line 28-30]

Level of interest: An article of importance in its field

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