

Author's response to reviews

Title: Vitamin E supplementation and pneumonia risk in males who initiated smoking at an early age: effect modification by body weight and dietary vitamin C

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Author's response to reviews: see over

Vitamin E supplementation and pneumonia risk in males who initiated smoking at an early age: effect modification by body weight and dietary vitamin C

By Harri Hemilä and Jaakko Kaprio 20 Oct 2008

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Replies to reviewer's comments (22 Aug 2008)

and

Description of changes in manuscript below the Replies

Dear Sirs,

Thank you very much for the reviewer comments that we received 22 Sep 2008.

Please, find here our replies to the reviewer comments.

Please, find our revised manuscript.

Yours,

Harri Hemilä

Jaakko Kaprio

Replies

HH+JK: Our replies are in ordinary text, whereas *the reviewer's comments are in italics*. We restrict to the parts of the reviewer's text that require comments by us.

Major Compulsory Revisions:

...

I believe their analysis needs to be redone with adjustment for important predictors of pneumonia risk before it can be considered for publication.

HH+JK: We agree that in tables 3 and 4 the subgroups get rather small. We calculated the adjusted RR and CI values to those two tables and replaced the unadjusted values with them. However, there are no substantial differences between the unadjusted and adjusted values. Nevertheless, this change prevents speculations that imbalance between the trial arms in those subgroups might lead to inappropriate estimates.

In this version, we still kept the Table 2 calculations unadjusted, because the subgroups are large. The smallest subgroups in Table 2 are the light and heavy weight participants, for which Tables 3 and 4 give the adjusted values. Thus, the reader has both adjusted and unadjusted estimates for these subgroups. If the editor considers that Table 2 should also be changed to adjusted estimates, we will do so.

.....

I am puzzled by the decision to further analyze this issue in the same dataset, rather than attempting to validate the finding in another study population, but perhaps this was not possible. It is true that a significant interaction was found

between age at smoking initiation (as a continuous variable) and vitamin E supplementation group in the Chest 2004 paper.

HH+JK: We are **not** analyzing the same issue in this manuscript.

In our Chest 2004 paper:

First, we found significant first-level interaction between the age participants started to smoke and vitamin E effect – so that vitamin E was beneficial for those who started to smoke at later age.

Second, among the participants who started to smoke at later age (n=7469), we found statistically significant second-level interactions. We found that vitamin E was beneficial to those who smoked less than a pack at baseline (no benefit for those smoking pack or more at baseline). We also found that vitamin E was beneficial for those who quit smoking during the follow-up (no benefit for those who continued). Thus, these second-level interactions found that vitamin E was beneficial for those who were least exposed to smoking, and that is the same direction we found in the first-level interaction: initiating to smoke at later age also means less exposure to smoking.

The number of participants in the ATBC Study is so large (n=29133) that it made the exploration of second-level interactions in the subgroup of 7469 participants reasonable, and the findings were qualitatively consistent with the first-level interaction.

When we see that 7469 participants is a large enough subgroup to analyze second-level interactions, it is justified to study second-level interactions in the complementary subgroup of 21657 participants – even more so, because this subgroup is three times as large.

However, from the point of view of biology, it did not seem reasonable to study primarily the role of smoking as a second-level interaction in this subgroup of participants who started smoking at early age. All ATBC Study participants were smokers and those who started to smoke at early age were obviously heavily exposed to smoking over all their life and it did not seem reasonable to copy the logic of the Chest 2004 paper to this subgroup. Thus, we are **not** *“further analyzing the same issue in the same dataset.”*

Instead, we asked that - if the 14% difference between vitamin E and no-vitamin E participants reflects a biological effect (instead of being a statistical artifact) - where would we expect the greatest effect. We hypothesized that the fixed dose might cause greater effect in participants with lowest body weight.

Weight is a second-level interaction, but because we have such a huge number of participants in the subgroup (n=21657), we were able to explore even third-level interactions and we found interaction with dietary vitamin C in both weight extremes, and this is the same variable which interacted with the effect of vitamin E on tuberculosis incidence in another ATBC study analysis.

If a large number of subgroup analyses are carried out, they inevitably lead to “significant” differences purely by chance, but the restriction of vitamin E effect to the weight extremes and the consistency of dietary vitamin C as a statistically significant modifier in both weight extremes show a logical structure which is not easy to explain as a result of multiple comparisons.

Because baseline smoking was important in the Chest 2004 analysis, we analyzed the role of smoking in the current study [our Results: “Level of smoking at baseline and leisure time exercise did not significantly modify the effect of vitamin E supplementation in the low or high body-weight subgroups (data not shown).”] but that is a side path in the current paper and we did not put the figures to Tables 3 and 4.

Thus, “*further analyze this issue in the same dataset*” is not a valid comment. In this manuscript we have very different approach compared with the Chest 2004 paper.

The reviewer found an inconsistency in our Chest 2004 paper. Pneumonia cases in the subgroup of those who initiated smoking by 15 years (n=180) and in the subgroup initiating between 16 and 20 (n=522) sum up to 702.

The correct number of pneumonia cases in those who initiated by 20 years was 701 (which is the figure of the current manuscript too).

The error of one case comes from the “15 years or younger” group which included one pneumonia case with missing “age of smoking initiation”. Thus the correct figure is 179 pneumonia cases in the subgroup of those who initiated smoking by 15 years. Such an error is unfortunate, but it has no relevant effect on the estimate and confidence interval (corrected RR 1.06, 95%CI 0.79-1.42).

However, it is not clear to me why this analysis focuses on the non-significant increase in relative risk among smokers who initiated at a younger age, and attempts to find evidence for a causal relationship indicating harm of vitamin E. The evidence for the increase in pneumonia risk (again, 1.14, 0.98-1.32) was weak enough in the previous analysis that this possible harm was not even commented on in the Chest publication.

HH+JK: See above.

The Chest 2004 paper was already quite long: 9 printed pages. Because of the space limits of journals and our own time limitations, it was not possible to carry out proper analyses of the “early smokers” for the 2004 report.

We do not quite understand the reviewer’s question “*why ... attempts to find evidence for a causal relationship*”.

One of the primary goals of science is to search for causal relationships.

If there is 14% difference in the average incidence of pneumonia, we consider it important to examine, whether the difference is caused by random variation or by a smaller group in which vitamin E causes greater than 14% harm while the majority might not be harmed.

The relevance of subgroup analysis is not based on the statistical significance of the effect in a group. In the Chest 2004 paper we found no overall effect, yet the interaction with “age at smoking initiation” was highly significant (p=0.0007) and justified further exploration in which we found interactions with other measures of smoking.

Similarly, the “*the non-significant increase in relative risk*” is no counterargument to our current subgroup analyses of the 21657 participants.

More specific comments on this paper include the following:

1. The background sections in both the abstract and introduction do not adequately present the rationale for this study, nor do they clearly relate the

hypothesis to the findings of the prior study and put the current paper into the context of the previous analysis.

2. The restriction of this analysis to a subset of the entire trial dataset is not clearly presented, in terms of proportion of participants and of pneumonia cases included, as well as any differences between this group and the excluded participants.

HH+JK: We described the rationale above. We cannot describe the rationale in Introduction in detail, but we rewrote parts of it.

3. Randomization for the ATBC study was not stratified based on age at initiation of smoking, and it is quite possible that there are some imbalances within the subgroup due to chance alone, especially with respect to important predictors of pneumonia risk. It has been recommended that “comparability of treatment groups for prognostic factors should be checked within subgroups” (please see Rothwell PM, Subgroup analysis in randomized controlled trials: importance, indications, and interpretation. Lancet 2005; 365: 176-86). I strongly feel that a table 1 should be included to examine the balance of the two groups for important predictors of pneumonia risk. In addition, both adjusted and non-adjusted results should be presented for the current analysis, including all predictors found to be significantly related to pneumonia risk in previous study. Confounding due to differences in strong predictors within this subgroup is quite possible.

Our current Table 1 describes where the participants of this analysis come from in general, but the purpose is not to compare the intervention groups.

This 21657 participant subgroup is 74% of all 29133 ATBC study participants. Although “*the ATBC study was not stratified based on age at initiation of smoking*” the 74% of ATBC population is such a huge population that it is not reasonable to assume imbalance between vitamin E and no-vitamin E groups.

Here are some examples of the vitamin E vs. no-vitamin E comparison within the current 21657 participants:

	Vitamin E	no-vitamin E
Age (yr)	57.64	57.56
Cigarettes (/day)	20.95	20.85
Alcohol (g/day)	18.70	18.85
Coffee (ml/day)	617.28	617.30
Unemployed	26.26	26.21

Among the variables above, the largest difference is in alcohol, in which there is 0.8% difference in the mean values. Thus, there is no reason to assume that excluding 25% of ATBC study participants would have generated biased intervention groups.

Reviewer’s concern may be valid for the smaller subgroups, but as mentioned above, we recalculated the Tables 3 and 4 again, adjusting for available risk factors for pneumonia. Although the balance is more relevant issue in the smaller subgroups, it is technically impossible to present tables to compare the distributions of all subgroups in Tables 3 and 4.

“both adjusted and non-adjusted results should be presented for the current analysis”

We think that both values together would rather confuse the reader (two sets of RRs and Clis), than increase their understanding. We mention in text that there are no substantial differences between the adjusted and unadjusted values, and that would seem close to optimal way to present the issue to readers.

4. Figures should include confidence intervals and number at risk in each group, to display the uncertainty in the data. Time-to-event plots comparing treatments by subgroups can mislead one into exaggerating the evidence of a subgroup effect.

HH+JK: We tested plotting of two survival curves with confidence intervals. It seems that STATA does not have an option to plot the cumulative hazards and its confidence intervals for two groups to the same figure. See at the bottom of this reply. Based on the plots at the bottom, it would seem that a plot with confidence interval for two groups would be difficult for a reader to understand.

The purpose of our survival curves is to show the time-distribution. It is possible that there are many cases clustered immediately after vitamin E supplementation is initiated, or at late phases of the follow-up.

The accuracy of the estimates (the CIs) are given for the proportional hazard regression models. We also give the p-values of the logrank test for the comparison of the curves, which describes whether random variation can explain the differences between the groups.

Thus, the purpose of our survival curves is different compared with the regression models and logrank tests. Confidence intervals for the survival curves would confuse the reader, while they have available measures of accuracy (CI) and statistical significance (log rank) even without the additional 4 lines per a plot.

5. In general, the paper should avoid use of terms like “harm of vitamin E” and “increased risk” definitively and be much more cautious in interpretation of the associations identified and conclusions drawn in this exploratory analysis. Post hoc observations should be treated with skepticism irrespective of their statistical significance. The authors do mention their multiple tests of interaction as a limitation, but additional caution due to the limitations I have pointed out is also warranted.

HH+JK: As we describe in Discussion, evidence that that vitamin E can be harmful to some population groups is not limited to our current subgroup findings. Graat et al. found in the Netherlands that vitamin E made respiratory symptoms of elderly people significantly more severe (up to $p=0.009$ in the direction of harm). In earlier analyses of the ATBC study, we found subgroups in which vitamin E increased the risk of tuberculosis and the common cold.

Furthermore, the analysis of harm and benefit are not symmetric. We may require firm evidence of benefit before a new intervention in medicine is taken into wide use. However, it is not reasonable to require firm evidence of harm for discouraging the use of ineffective intervention.

For example, in our group of 21657 participants, the confidence interval is consistent with no difference between vitamin E and no-vitamin E (“nonsignificant” difference). However, the lower limit of confidence interval ($=0.98$) refutes the possibility that vitamin E

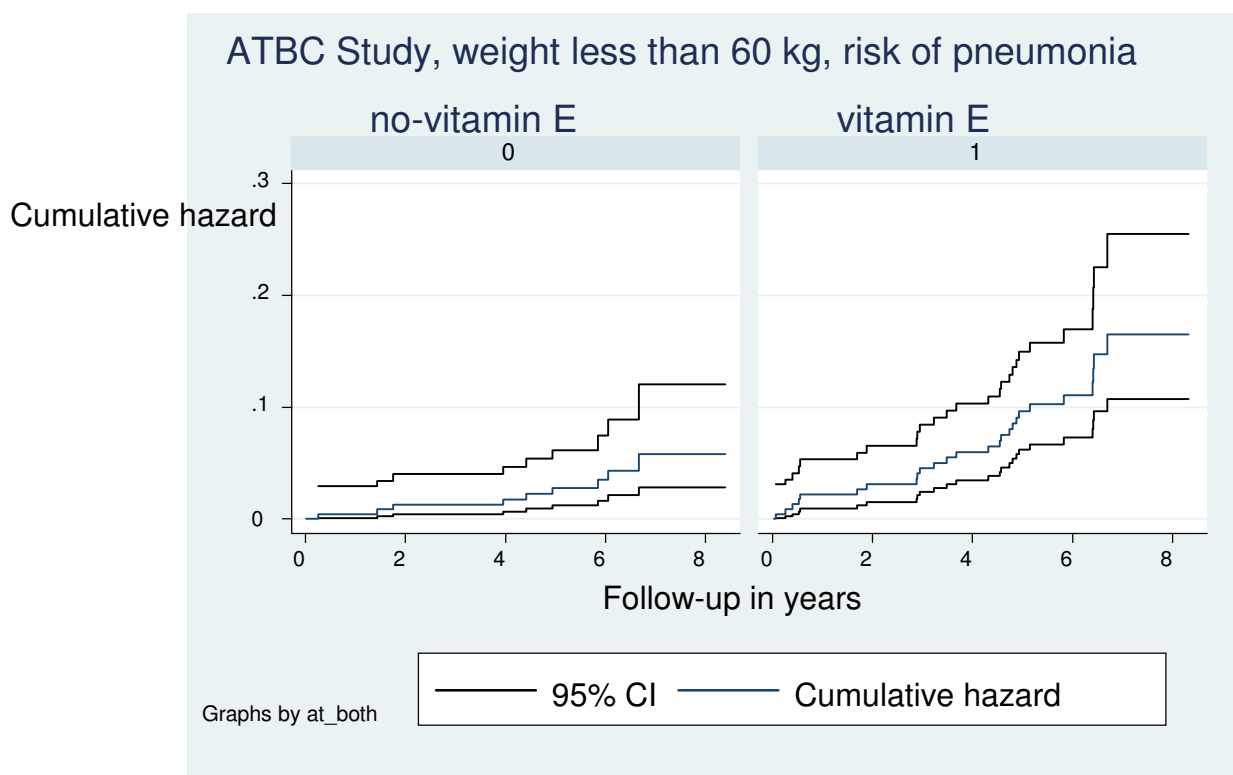
supplementation would reduce the risk of pneumonia by more than 2% in males who start to smoke early in their life. Given the common proposals that vitamin E improves the immune system, this lower limit of confidence interval justifies discouraging vitamin E for “improving immune system”, although the difference is not statistically significant.

We do not know to which specific part of our text the reviewer refers to. In our Discussion we refer to other studies which have found harm and in our Abstract we implicitly refer to the wider literature than the current study when proposing that “Our findings should increase caution towards taking vitamin E supplements.”

We reread our manuscript with reviewer’s comment in mind, but we do not consider that the claims of potential harm are too strong. If the editor considers that, in some parts, the current version is too strong in suggesting that vitamin E may be harmful for some population groups, we are ready to revise our text, given explicit parts of the text.

The STATA program does not seem to have the option to present two cumulative hazard curves in the same figure with their confidence intervals. These plots below show the problem.

Although different types of lines can be used to mark the two groups and the confidence intervals, the set of six lines in one plot would be very difficult for a reader to understand. Confidence intervals for cumulative hazard plots seem to be at their best when there is only one group for which we wish to have an impression of accuracy.



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Changes in the manuscript

Based on the reviewers' comments, we made the following changes to the manuscript.

Introduction:

We reorganized and rewrote paragraphs 4 and 5 to give a more unambiguous background for the current subgroup analyses.

Statistical methods:

Third paragraph: we describe the adjustment of Cox models.

Results:

Second paragraph at the end: we justify the adjustment of Cox models in Tables 3 and 4.

Table 2:

Footnote: We added two tests for heterogeneity in the weight groups.

Tables 3 and 4:

Footnote: we added the description of adjustment.

Tables: we changed the number of participants, estimates and confidence intervals to the adjusted comparisons.