Antioxidants are not identical and their effects are not uniform over the population, 24 August 2009

Summary

Antioxidants are not identical and their effects are not uniform over the population

Harri Hemilä, MD, PhD
Department of Public Health, University of Helsinki, Helsinki, Finland. e-mail harri.hemila@helsinki.fi

Submitter agrees with the default conflict of interest statement: I certify that I have no affiliations with or involvement in any organization or entity with a financial interest in the subject matter of my feedback.

Reply

Dr. Harri Hemilä wrote:

There are two fundamental problems with the review by Bjelakovic and coworkers. First, the authors are combining apples and oranges. Second, the authors ignore the evidence indicating that vitamin E effect is not uniform over the population.

First, let us consider an analogy. If a researcher is interested in the effect of antibiotics on mortality caused by infections, and combines all antibiotics to a single broad category of 'antibiotics', and pools all 'antibiotic trials' together, people with basic background in clinical microbiology would consider such a project silly. Different antibiotics kill different bacteria, there is geographic and social group variation in the occurrence of pathogenic bacteria, the usage of antibiotics generates resistant strains, etc., etc. The biology of antibiotics is very complex. It is obvious that a single universal estimate for 'antibiotic effect' is meaningless.

Antioxidants are also a heterogeneous group. Vitamin C is water soluble, vitamin E is fat soluble, selenium is an inorganic element, beta-carotene is not an essential nutrient, etc. Moreover, in model systems small-molecule antioxidants are oxidized at different speeds. For example, activated neutrophils in plasma first oxidize vitamin C, whereas the oxidation of urate starts only after vitamin C has been consumed, and the level of vitamin E remains virtually unchanged [1]. From the point of view of biology, there is no basis to consider that all antioxidants are similar enough to justify the pooling of all trials with compounds belonging to the broad category of 'antioxidants'. In a proper analysis, each antioxidant should be analyzed specifically.

- Response:

We thank Dr Hemilä for the comments. We agree that 'antioxidants' are a heterogeneous group of substances. Vitamin E and the other antioxidant supplements may have different effects in different populations. Accordingly, we have accounted for the statistical heterogeneity in our analyses. Several potentially interesting subgroup analyses may be considered, but the included trials do not report the necessary data. When performing systematic reviews, the decision to split or lump a heterogeneous group of trials may be difficult. Splitting trials into various subgroups may provide more focused answers, but will also increase the risk of spurious results. Lumping a heterogeneous group of trials provides a less focused answer, but may increase the external validity. In the present review, we decided to focus on antioxidants supplements to provide an overall picture of the potential effects. Our results provide information that may form basis for future trials and reviews.

We have analysed antioxidant supplements combined (beta-carotene; vitamin A; vitamin C; vitamin E; selenium) as well as individually.

Dr. Harri Hemilä wrote:

Bjelakovic and coworkers label vitamin A as an antioxidant. Halliwell and Gutteridge write in the latest edition of their monograph, that "vitamin A can do the same [scavenging some oxidants, as beta-carotene does] but no data exist supporting such a role in vivo?" [2 p. 177]. Of course, Halliwell and Gutteridge may be wrong, but given the depth of their familiarity with the antioxidant literature, Bjelakovic and coworkers should give explicit reference(s) to reject that statement when labelling vitamin A as an antioxidant. Most biomolecules are oxidized by hydroxyl radical, but it is not reasonable to thereby label all of them as antioxidants. Halliwell and Gutteridge suggest that the definition of "antioxidant" should require that it delays, prevents or removes oxidative damage [2 p. 80-81]. Thus, a systematic review focusing on antioxidants should consider and define what is meant by an "antioxidant". Bjelakovic writes that "there are pros and cons in the literature about vitamin A being antioxidant", which in my opinion is not a scientific argument to justify labelling vitamin A as an antioxidant. Furthermore, Bjelakovic and coworkers write in Discussion that "recent research revealed that vitamin A can cause oxidative damage to DNA". Thus, there is lack of logic when claiming that vitamin A has been shown to be a pro-oxidant, but include it in the review restricted to antioxidants on the basis of conflicting opinions.

- Response:

Based on previous evidence, we have classified vitamin A as an antioxidant supplement. The potential antioxidant and pro-oxidant potentials of vitamin A are clarified in the discussion section of our review. We kindly refer the reader to this discussion.

Multivitamins/multiminerals (containing vitamin A) are the most commonly used dietary supplements. What is important is the effect of vitamin A on mortality irrespective of specific biological effect.

Dr. Harri Hemilä wrote:

Analyzing trials by the specific biochemical substance that is tested would make the review much more logical. If a review focuses on, say, vitamin A and mortality, there is no need to firmly decide whether vitamin A is an antioxidant or not. The problem of defining 'antioxidant', and deciding whether some of them is pro-oxidant under certain condition is avoided if the substances are analyzed by the biochemical definitions. In such an approach, antioxidant and pro-oxidant concepts could be left to the Discussion section for giving plausible biological explanations for the observed effects.

An important goal in modern biomedicine is specificity. The strength of the randomized trial is that the difference between the trial groups can be specifically attributed to the intervention that is being tested. However, when the intervention varies from a single antioxidant to the combinations of diverse antioxidants, and includes 11 trials in which "participants were supplemented with different mixtures of antioxidants as well as with vitamins and mineral without antioxidant properties", we lose specificity because of the apples and oranges problem.

- Response:

We agree that specificity is an important goal. This is why we report meta-analyses of the selected five antioxidant supplements together (beta-carotene; vitamin A; vitamin C; vitamin E; selenium) as well as meta-analyses of them individually (beta-carotene; vitamin A; vitamin C; vitamin E; selenium). Our intention by also using meta-analyses of the individual supplements was to evade the critic of mixing 'apples and pears' or 'apples and oranges'.

Dr. Harri Hemilä wrote:

In Table 6, Bjelakovic and coworkers calculate the specific effect of vitamin A (95% CI for the RR: 0.84 to 1.68; N=2406) and vitamin E (95% CI for the RR: 0.98 to 1.05; N=41341), but these specific effect estimates are hidden from the reader. In the Discussion, Bjelakovic and coworkers state that "beta-carotene, vitamin A, and vitamin E given singly or combined with other antioxidant supplements significantly increase mortality", which is false and misleads those readers who skip Table 6 and only look at the Discussion. The above confidence intervals show that vitamins A and E singly do no significantly increase mortality. In the Abstract, Bjelakovic does not give the specific estimates for vitamins A and E, but gives RR estimates based on studies with scores of other antioxidants including beta-carotene, which has been known to increase mortality since the publication of the ATBC and CARET trials. A reader of the Abstract cannot figure out that the given RR-values don't tell us anything about the specific effects of vitamins A and E. Thus, even the Abstract is misleading.

- Response:

As described in the methods section, we described our results of the overall, subgroup, and sensitivity analyses. We are surprised that Hemilä accuses us from hiding any information the information from the random-effects model analyses were clearly presented in Table 5 and the corresponding analyses from the fixed-effect analyses were presented in Table 6. Now Hemilä is especially interested in certain subgroup results. Such data may be misleading as they may not have the necessary power and precision. The data we present in our abstract are those having the best power and precision and hence represent findings with most external validity.

Second, when Bjelakovic and coworkers first published the review in JAMA, I pointed out that the effect of vitamin E on respiratory infections was heterogeneous in the large scale ATBC Study [3,4]. Vitamin E had no overall effect on the incidence of the common cold or pneumonia, but the effects were significantly modified by age and smoking [5,6]. Although heterogeneity in the effect on respiratory infections does not directly imply that the effect on mortality must be heterogeneous, such a possibility should not be dismissed. If the effect of vitamin E is heterogeneous, then a single estimate for effect can be meaningless. However, Bjelakovic ignores this issue.

Motivated by our findings on respiratory infections, we analyzed the effect of vitamin E on the mortality of ATBC participants and found strong evidence that the effect of vitamin E on total mortality was also heterogeneous [7,8]. Vitamin E had no effect on those who had low dietary vitamin C intake; however, among those who had high dietary vitamin C intake, vitamin E increased mortality in young and decreased mortality in old participants. Close to half of the participants fell to those groups in which vitamin E effect was inconsistent with the average effect of the whole study population. When the average effect of a large trial is misleading for half of the study participants, it seems obvious that calculating and presenting a single universal 'estimate for vitamin E effect' is an unsound approach.

Although heterogeneity in vitamin E effect on mortality does not directly imply that the effect of vitamin C and beta-carotene must be heterogeneous, such a possibility should not be ignored. In fact, we can even turn the argument around. Given the strong evidence that vitamin E effect is heterogeneous, why should we accept such a premise that the effects of other antioxidants are uniform over the population. If we assume that the effects of antioxidants are heterogeneous, further studies should try to identify and characterize the subpopulations where the antioxidants might be beneficial, rather than calculating a fictionally accurate average effect on all people. Lack of uniformity in vitamin C effect is suggested by the interaction between vitamin E supplementation and dietary vitamin C intake.

Although dietary vitamin C has a high level of correlation with other substances in fruit and vegetables, the other substances did not explain the modification of vitamin E effect in the ATBC cohort [7].

- Response:

Dr Hemilä reports some interesting subgroup analyses from a randomised trial. The data suggest a possible beneficial effect of vitamin E given in combination with vitamin C seen in certain populations. Although we cannot exclude such an effect, we are unable to analyse the question as only trial-level data were available for our meta-analyses. In our review, we have clarified that the effect of antioxidant supplementation might not be uniform across the population.

Bjelakovic and coworkers write in their Discussion that "our analyses had little trial heterogeneity. This increases the trustworthiness of our findings". I cannot see any justification for such an argument. If there is a strong premise that the effect of, say, vitamin E should be uniform over the population, in such a case observing little heterogeneity is consistent with our expectations. However, as noted above, there is no basis for such a premise in general, and in the case of vitamin E it was firmly refuted [7]. The level of heterogeneity is no measure of 'trustworthiness'.

Bjelakovic and coworkers state in their Discussion that "adoption of the random-effects model in meta-analysis permits extension of inferences to a broader population of studies than the fixed-effect model does", which is incorrect. If there is heterogeneity, we do not know to whom the calculated overall estimate applies, and this problem does not disappear by using the random-effects model. In the random-effects model the confidence interval is wider, but that does not help us to understand what are the characteristics that modify the effect: to whom there is effect and to whom not. When there is evidence of heterogeneity, the main focus should be on trying to understand any sources of heterogeneity that are present [9].

- Response:

In systematic reviews, different sources of intertrial heterogeneity may exist including clinical, methodological, and statistical heterogeneity.[17] **In our review, we found little evidence of intertrial heterogeneity in our meta-analyses. In our meta-regression analyses, the risk of bias and the type of antioxidant supplement were the only significant predictors of intertrial heterogeneity. In the trials with a low risk of bias, the antioxidant supplements significantly increased mortality (RR 1.05, 95% CI 1.02 to 1.08).** [18] **We have discussed differences between random-effects and fixed-effect models of meta-analysis in the section Discussion.**[3]

Bjelakovic and coworkers also conclude that the effect of antioxidants is uniform over the duration of supplementation: "we found no significant effect of treatment duration on our results" (Discussion). Our analysis of the individual-level data of the ATBC study refuted also this conclusion. In young participants who had high dietary vitamin C intake, vitamin E supplementation had no effect over the first 3.3 years, but thereafter increased mortality by 38% [7]. Adding the two different vitamin E effects significantly improved the Cox model (P=0.007). Correlation of treatment effect with the average duration of supplementation at the trial level is too crude a method to examine the time dependency of supplementation effects. In epidemiology, 'ecological fallacy' means thinking that relationships observed for the averages for groups necessarily hold for individuals. Thus, Bjelakovic's conclusion that treatment duration has no effect on the effect of antioxidant supplementation is an example of the ecological fallacy.

- Response:

We found a number of trials assessing similar intervention regimens to the one assessed in the ATBC trial. Based on our meta-regression analyses stratified by the intervention regimen, the duration of supplementation was not a predictor of the estimated intervention effect.

Finally, Bjelakovic and coworkers were ambitious when covering all antioxidants plus vitamin A trials. Such wide coverage requires lots of work and easily leads to errors in the extraction of data, and to the lack of time to read the papers and learn the context of the trials. As a reflection of this problem, Bjelakovic and coworkers wrote half-a-page erratum to their JAMA paper [10]. Nevertheless, in the 2008 version of the Cochrane review Bjelakovic still includes the Chandra 1992 trial [11] in their analysis, even though it had been shown to be fabricated several years earlier. The story should be familiar to everyone who follows the major journals [12-15]. In the reference list, under the citation of the Chandra 1992 report, Bjelakovic cites the Lancet letter [12]. Apparently, Bjelakovic and coworkers lacked time to read the Lancet letter to see that there would have been good reasons to exclude the 1992 study from analysis.

- Response:

The most commonly used dietary supplements in adults are multivitamins/multiminerals.[19] **The daily use of vitamin A, C, and E also increased significantly during the last decades. Assessing the effects of these interventions is, therefore, important. We are aware that there may be limited bias control in the trial by Chandra and co-workers, but had to include the trial in our overall assessment. We did not include the trial by Chandra that was retracted in 2005 (Chandra RK. Effect of vitamin and trace-element supplementation on cognitive function in elderly subjects. Nutrition 2001;17:709-12). The second trial by the same group, published in Lancet in 1992 was included in our meta-analysis. Excluding this trial with 96 participants and 2 deaths in the placebo arm did not change our overall results.**

Bailar criticized the meta-analysis approach in general and gave examples of severe errors in five influential meta-analyses [16]. In particular, Bailar criticized the 'job-shop' approach: a group of researchers picks a topic, rushes to collect trials and pools their results,

without making themselves familiar with the biology and other relevant context of the topic. Lack of considering the differences between antioxidants, labelling vitamin A as an antioxidant (while simultaneously claiming that vitamin A is a pro-oxidant), ignoring the evidence of heterogeneity in vitamin E effect, the large number of errors in the first version of the meta-analysis [10], the inclusion of the Chandra 1992 trial; all these indicate to me that there is a severe 'job-shop' type of problem in Bjelakovic's review.

- Response:

We included vitamin A, vitamin C, vitamin E, beta-carotene, and selenium based on their proven antioxidant function. These antioxidants were chosen after extensive discussion of the antioxidant literature. Moreover, these antioxidants were accepted following extensive peer reviewer and editorial assessments of our Cochrane Hepato-Biliary Group protocol published in 2003. [20] **As this protocol covered more than cancers in the liver and biliary tract - but focused on all gastrointestinal cancers - we had our protocol quality assessed and approved by the Editorial Teams of The Cochrane Upper Gastrointestinal Diseases Group, The Cochrane Inflammatory Bowel Diseases Group, and The Cochrane Colorectal Cancer Diseases Group as well as The Cochrane Hepato-Biliary Group. So quite contrary to what Dr. Hemilä seems to imagine, we find the 'job shop' description the least well description of the extensive process we went through before assembling and extracting any data. Our present systematic review is an extension of this 2003 protocol now focusing on all-cause mortality.**

There is evidence that not only vitamin A but also vitamin C and beta-carotene possess pro-oxidant function.[21,22]

I do not disagree with Bjelakovic and coworkers about the main conclusions. So far, there is no good evidence indicating that ordinary people would benefit from taking antioxidant supplements for the purpose of reducing mortality. This conclusion can be reached by reading the major trial reports separately, without calculating a fictional pooled antioxidant effect. In this respect, pooling of the results does not give us any additional understanding. Although the evidence of heterogeneity in the vitamin E effect on mortality is strong [7], I do not think that it justifies practical conclusions yet. Rather, the complexity encourages caution in drawing conclusions and patience in waiting for further research.

- Response:

Based on the results of recently completed (PHS II; WACS)[23,24] **and prematurely terminated randomised trials (SELECT)**[25] **as well as earlier meta-analyses**[26,27] **we do not suggest further randomised trials testing the effect of antioxidant supplementation for primary prevention or secondary prevention. It may well be that there may be subgroup of patients with active diseases that may potentially benefit from certain antioxidants but that need proper assessments in randomised clinical trials as well as systematic reviews of such trials.**

Bailar commented that meta-analysis can aid in filling in the second and third decimal places once the questions are clear but it is a poor tool for developing new concepts, new hypotheses [16]. The Bjelakovic and coworkers meta-analysis implies that there is no justification for further research on vitamin E and mortality because the particularly narrow confidence interval (0.98 to 1.05) firmly rejects any substantial benefits. In contrast, our analysis of the ATBC Study suggests a path that should be explored: does the combination of vitamins E and C improve the health of some subpopulations of elderly males. In this respect, my conclusions significantly diverge from those of Bjelakovic. Therefore the two problems discussed above are fundamentally important.

- Response:

We agree that dogmas like the one that antioxidants, especially vitamin E supplementation, might be beneficial for human population, is sometimes very difficult to disaffirm.

In recent years, several major studies of vitamins and supplements have produced disappointing results. One recent example is the Selenium and Vitamin E Cancer Prevention Trial (SELECT) originally scheduled to end in 2011. SELECT was terminated in October, 2008 over a disproportionally high incidence in prostate cancer in participants in the trial who were taking vitamin E.[25]

References:

1. Frei B, Stocker R, Ames BN. Antioxidant defences and lipid peroxidation in human blood plasma. *Proc Natl Acad Sci USA* 1988; 85:9748-52.
http://www.pnas.org/content/85/24/9748

2. Halliwell B, Gutteridge JMC. *Free Radicals in Biology and Medicine*, 4th ed. Oxford University Press, Oxford, 2007.

3. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Mortality in randomized trials of antioxidant supplements for primary and secondary prevention: systematic review and meta-analysis. *JAMA* 2007;297:842-57. http://dx.doi.org/10.1001/jama.297.8.842

4. Hemilä H. Antioxidant supplements and mortality. *JAMA* 2007;298:401. http://dx.doi.org/10.1001/jama.298.4.401-a

5. Hemilä H, Virtamo J, Albanes D, Kaprio J. The effect of vitamin E on common cold incidence is modified by age, smoking and residential neighborhood. *J Am Coll Nutr* 2006;25:332-9.
http://www.jacn.org/cgi/content/abstract/25/4/332

6. Hemilä H, Virtamo J, Albanes D, Kaprio J. Vitamin E and beta-carotene supplementation and hospital-treated pneumonia incidence in male smokers. *Chest* 2004;125:557-65.http://dx.doi.org/10.1378/chest.125.2.557

7. Hemilä H, Kaprio J. Modification of the effect of vitamin E supplementation on the mortality of male smokers by age and dietary vitamin C. *Am J Epidemiol* 2009;169:946-53.
http://dx.doi.org/10.1093/aje/kwn413
8. Hemilä H. Vitamin E is likely to affect mortality even at low doses. *Clin Trials* 2009;6:392-3.
http://dx.doi.org/10.1177/1740774509340211
9. Thompson SG. Why sources of heterogeneity in meta-analysis should be investigated. *BMJ* 1994;309: 1351?5. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2541868
10. Bjelakovic G. Corrections. *JAMA* 2008;299:765-6. http://jama.ama-assn.org/cgi/content/full/299/7/765
11. Chandra RK. Effect of vitamin and trace-element supplementation on immune responses and infection in elderly subjects. *Lancet* 1992;340:1124-7. http://dx.doi.org/10.1016/0140-6736(92)93151-C
12. Carpenter KJ, Roberts S, Sternberg S. Nutrition and immune function: a 1992 report. *Lancet* 2003;361:2247-8. http://dx.doi.org/10.1016/S0140-6736(03)13755-5
13. White C. Three journals raise doubts on validity of Canadian studies. *BMJ* 2004;328:67.
http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=314042
14. Smith R. Investigating the previous studies of a fraudulent author. *BMJ* 2005;331:288-91.
http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=1181274
15. Meguid MM. Retraction [of a Chandra study based on the Lancet 1992 data]. *Nutrition* 2005;21:286.
http://dx.doi.org/10.1016/j.nut.2004.12.002
16. Bailar JC. The practice of meta-analysis. *J Clin Epidemiol* 1995;48:149-57. http://dx.doi.org/10.1016/0895-4356(94)00149-K.
17. Higgins JPT, Green S (editors). *Cochrane Handbook for Systematic Reviews of Interventions* Version 5.0.1 [updated September 2008]. The Cochrane Collaboration, 2008. Available from www.cochrane-handbook.org.
18. Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C. Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. *Cochrane Database of Systematic Reviews* 2008, Issue 2. Art. No.: CD007176. DOI:10.1002/14651858.CD007176.
19. Radimer K, Bindewald B, Hughes J, Ervin B, Swanson C, Picciano MF. Dietary supplement use by US adults: data from the National Health and Nutrition Examination Survey, 1999-2000. *Am J Epidemiol* 2004;160:339-49.
20. Bjelakovic G, Nikolova D, Simonetti R. Antioxidants for preventing gastrointestinal cancers (protocol for a Cochrane Review). The Cochrane Database of Systematic Reviews 2003, Issue 2.
21. Podmore ID, Grifiths HR, Herbert KE, Mistry N, Mistry P, et al. Vitamin C exhibits pro-oxidant properties. *Nature* 1998;392:559.
22. Paolini M, Abdel-Rahman SZ, Sapone A, Pedulli GF, Perocco P, Cantelli-Forti G, et al. Beta-carotene: a cancer chemopreventive agent or a co-carcinogen?. *Mutation Research* 2003;7719:1-6.
23. Sesso HD, Buring JE, Christen WG, Kurth T, Belanger C, MacFadyen J, et al. Vitamins E and C in the prevention of cardiovascular disease in men: the Physicians' Health Study II randomized controlled trial. *JAMA* 2008;300:2123-33.
24. Cook NR, Albert CM, Gaziano JM, Zaharris E, MacFadyen J, Danielson E, et al. A randomized factorial trial of vitamins C and E and beta carotene in the secondary prevention of cardiovascular events in women: results from the Women's Antioxidant Cardiovascular Study. *Arch Intern Med* 2007;167:1610-8.
25. Lippman SM, Klein EA, Goodman PJ, Lucia MS, Thompson IM, Ford LG, et al. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). *JAMA* 2009;301:39-51.
26. Miller ER 3rd, Pastor-Barriuso R, Dalal D, Riemersma RA, Appel LJ, Guallar E. Meta-analysis: high-dosage vitamin E supplementation may increase all-cause mortality. *Ann Intern Med* 2005;142:3746.
27. Vivekananthan DP, Penn MS, Sapp SK, Hsu A, Topol EJ. Use of antioxidant vitamins for the prevention of cardiovascular disease: meta-analysis of randomised trials. *Lancet* 2003;361:201723.

Contributors

Goran Bjelakovic,
Christian Gluud,
Lise Lotte Gluud
Rosa Simonetti,
Dimitrinka Nikolova.

Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases (Review)

Bjelakovic G, Nikolova D, Gluud LL, Simonetti RG, Gluud C

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