**Author's response to reviews**

**Title:** The effect of long-term homocysteine-lowering on carotid intima-media thickness and flow-mediated vasodilation in stroke patients: a randomized controlled trial and meta-analysis

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**Author's response to reviews:**

6th July, 2008

Dear Lolu,

Thank you very much for the opportunity to revise our manuscript. Please find the revised version attached. The changes made in response to the reviewers’ comments are listed below.

Petra Verhoef

**Discretionary revisions**

1. The manuscript title has been changed as suggested.
2. Information on the numbers included in VITATOPS and the expected reporting date have been included in the “Participants” section of the methods (p.5).
3. The abstract now mentions that the participants were a subgroup of the VITATOPS subjects

**Minor essential revisions**

1. That there were no baseline measurements of FMD and CIMT is now stated clearly at the beginning of the “Outcomes” section of the methods (p.6)
2. The method used to calculate the effect size (Cohen’s d) and the method of bias correction (Hedges) is now mentioned in the “Statistical analysis” section of the methods (p.8). (See also note below in response to Andrew Willan’s comments regarding the effect size calculations)
3. Figure 3 has been removed.
4. Unfortunately the software we used to generate the funnel plot is not flexible enough to change the size of the points according to study size. However, the
funnel plot is designed to indicate the relative size of the studies by plotting the
SE in the measurements against the weighted mean differences. The variance of
the measurement (FMD and CIMT) is assumed to be similar between studies,
but studies with smaller subject numbers tend to have larger standard errors
because the standard error is influenced by subject number.

Jan Auke Rauwerda

1. We recruited half of the subjects who were eligible to participate (173/343) and
decided to do an on-treatment analysis, because we wanted to maximise the
change of finding a treatment effect of B-vitamins. Therefore we considered it
reasonable to exclude data from subjects who were no longer taking the study
medication or had started taking vitamin supplements. We did not collect the
reasons for no response or decline, as our ethics approval stipulated that we
should not contact the non-responding subjects by telephone. I have included
some additional analysis of baseline characteristics of the participants and
non-participants in Table 1 (see also note below in response to Andrew Willan’s
comments about the non-response rate).

2. We included all VITATOPS subjects because we were primarily interested in
the effect of long-term B-vitamin treatment on the vascular measurements of
FMD and CIMT and we wished to maximise the number of subjects included.
There was no between-group difference in these outcomes in the sub-group of
subjects with a history of ischemic stroke.

3. I apologise for leaving out the statin data which is now included. Many thanks
for drawing my attention to this oversight.

4. We did not test the subjects for recent consumption of alcohol or tobacco or
coffee, but we did ask them when they last ate, drank alcohol or coffee or
smoked and relied on their honesty. Data from subjects who admitted to
consuming any of these substances within six hours of the study appointment
were excluded.

5. Intra-observer variability data are available for both FMD and CIMT
measurements and are now included in the paper.

6. The reviewer seems to have misunderstood the study design, as the study
was not stopped for two years at all. I hope the changes detailed above have
made the study design easier to understand.

7. We did not look specifically for adverse events in this study, although the
VITATOPS trial has been monitoring these. This section has been removed.

8. We have now included follow-up data on fruit and vegetable intake (Table 2).
These data were not collected at baseline.

Andrew R. Willan

Major Compulsory Revisions

1. Of the 532 VITATOPS subjects randomised between 1998 and 2003, only 343
subjects were eligible for inclusion in this sub-study, as the remainder had either
died, withdrawn from VITATOPS, moved away from Perth or were too unwell or disabled to attend an appointment at the hospital. Our ethics approval stipulated that we should not contact subjects who did not reply to the initial invitation letter, so we were unable to follow-up the non-responders. I have added an analysis and discussion of the baseline characteristics of the participants and non-participants in the Results section and Table 1 and more fully addressed the potential sources of bias in the “Limitations” section of the discussion (p.16).

2. The type I error rate initially used for sample size determination was two-sided. I have amended the post-hoc power calculations to a one-sided error rate. Many thanks for drawing my attention to this.

3. The p-values in Table 1 (now Table 2) have been removed.

4. In Table 2 (now Table 3) the p-values for “Baseline” and “Post-treatment” comparisons have been removed. I have not replaced the 95% confidence intervals with standard deviations as it is my understanding that log-normal distributions are best represented by a geometric mean with 95% confidence interval. I apologise if the reviewer was unable to identify the numbers in parentheses, but it was stated in the “Statistical analysis” section of the methods that: “Variables with log-normal distributions were log-transformed…..results for these variables are reported as geometric mean (95% confidence interval)” and also in the notes under the table: “Baseline and follow-up values are presented as geometric mean (95% confidence interval)”. Again, my apologies if this was not clear. I have replaced the change from baseline values with the adjusted mean treatment differences, standard errors and p-values from a GLM using the model indicated.


Discretionary Revisions

1. Many thanks for the suggestion, but I have not undertaken this additional analysis.

Thank you again for giving me the opportunity to revise this manuscript. Please don’t hesitate to contact me if you require any further information. I look forward to hearing from you in due course.

Kind regards,

Kathleen Potter