Reviewer's report

Title: Vitamin C and E supplementation does not affect heat shock proteins or endogenous antioxidants in trained skeletal muscles during 12 weeks of strength training

Version: 0 Date: 14 Apr 2017

Reviewer: Marco Mensink

Reviewer's report:

In this manuscript Cumming et al described the effects of resistance exercise training, with or without vitC/E supplementation on the acute stress responses to exercise and adaptation to training with regards to NFkB pathway, HSP's and antioxidant enzymes. Data from this study with regard to hypertrophy related signaling and muscle strength/mass were already reported earlier (Paulsen, 2014).

The study and its analysis are of good quality.

The limited sample size and power of the study, in particular with regard to the acute stress response, makes interpretation of the data hard, and implication limited. This is acknowledged by the authors, but is a severe limitation. Data on the number of samples analysed for the different genes/proteins is missing, and should be added.

The results didn't reveal any acute exercise or training effect on the measured genes/proteins, except for IkBa in the placebo group at t=100. So any conclusions on a blunting effect of supplementation (as hypothesized) seems not warranted. This lack of exercise/training effects should be acknowledge in the conclusion, and carefully considered when drawing conclusions about supplementation (L283-286).

Much of the pre-knowledge on HSP and antioxidant supplementation is derived from endurance exercise (training); Could the mode of exercise somehow be related to the lack of effect on HSP in the present study, besides the training status of the volunteers (L238-250)

Comments
L73. What were the results of the endurance training supplementation trial (Cumming 2014b)

L91-92. What was the training experience of the volunteers? How many years were the already active?. Additional data on for example 1RM would be informative. This is relevant regarding the comments in the discussion on the role of training status
L 99. Would love to see some information about the result of the program on strength and mass. Moreover presenting the results of the present manuscript at the background of results already published in the past (Paulsen 2014 a and c) would benefit the discussion. Sample size and power of the study seems large enough to detect effects of supplementation in other pathways (i.e. hypertrophy related signaling)

L102. Was 1RM re-assessed during the intervention, and the training protocol adapted?

L103. I don't understand the information between brackets (6-10RM); does it mean 6 to repetitions? At which % of the 1RM?

L105. How many exercises were performed?

In general: Although data from the present study were published earlier, some details about the study design would benefit the present manuscript (L98)

L 111. What were the characteristics of these 15 volunteers, and how many per group?

L119-133. I would suggest to describe the information on the supplements somewhat earlier in the method section, at least before the 'acute exercise session' section; as this makes it understandable why supplements were also provided during that session

L132. Did subjects use NSAID's or was this prohibited during the study? (as for other medication)

L136. Where the pre and post training biopsies taken in the fasted state? Or after breakfast, with or without supplements?

The baseline muscle biopsy samples during the acute session were taken postprandial (2h after the breakfast). Is this comparable to the pre and post training sample as is relevant for the data presented in figure 4 (pre-mid-post)

L139. How and where were the three samples taken in the acute study? Same or different incision? Proximal or distal, and distance?
L158. Details for HSP 27 are missing (->figure 4)

How many muscle biopsy samples could be analysed for the different groups/ timepoints? Was a sample available for each volunteer at each occasion? Indicate those numbers in the text/figure legends

Figure 1. I suggest to add a heading indicating the protein measured (IkBa). Like is done for the other figures. And indicate the significant effect at t=100 for placebo group

Figure 2 and 3: why not combine? As they contain same level of data (gene expression in acute study)

Figure 4: representative immunoblots for HSP 27 are missing; pleas indicate that P-value relates to difference between groups (and not over time).

L 211. How many subjects were in each analysis?

L 213. Also describe the results presented in Figure 5 in the main text

L236/237. How many biopsies were available?. This relates to earlier remarks I made. As the authors acknowledge the limited sample size and power, I would like to see numbers throughout the manuscript.

L245. What is the authors' definition of 'moderately'

L258. Can an estimation be given what power would have been needed according to the authors to draw more solid conclusions?
Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

Quality of written English
Please indicate the quality of language in the manuscript:

Acceptable

Declaration of competing interests
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?

6. Do you have any non-financial competing interests in relation to this paper?
If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

I declare that I have no competing interests

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal