Author's response to reviews

Title: Can supplementation with vitamin C and E alter physiological adaptations to strength training? Design and rationale.

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Author's response to reviews: see over
Reviewer #1

Authors: We would like to thank this reviewer for insightful and fair critique and recommendations. Your suggestions have improved the manuscript.

Major Compulsory Revisions
The major concern is regarding the design of the study in the following aspects.
1. First of all it should be clearly stated which measurements are done in each time-point (for example more complete information in Figure 2).

Authors: Thank you for pointing this out, we agree. Figure 2 is changed as requested.

2. In the “Test and measurements” section the methodology used for measuring most of the parameters is exhaustively described. However, the information regarding the blood and muscle samples as well as the description of the parameters measured in these samples is poor. We need to go to lines 176-181 to guess that antioxidants would be measured in these samples. But, for example, it is stated that about 18 ml of blood were taken. This is a lot of sample for measuring only endogenous antioxidants, vitamins C, E and glutathione. This should be clarified.

Authors: This a good point. We have added information of parameters and methods (line 415-421).

3. Methods used to perform the measurements in blood and muscle samples should be described as it is done for the other methods used.

Authors: Parameters and methods are now better explained in “Blood sampling and analyses” (line 400-421). Thank you for pointing this out.

4. How was the muscle soreness recorded? Which methodology was used? Clarify.

Authors: Soreness was simply rated on a scale from 0-10, as now described under “Training diary” (line 267-269).

5. It is indicated that the participants in the acute experiment were the same as in the long-term experiment. Were the participants randomized again for this experiment? Did the participants from the supplemented group in the long-term experiment belong to the supplemented group in the acute-experiment? The effects observed in the supplemented group in the acute experiment were due to acute or to the prolonged supplementation? Can you differentiate them?

Authors: Yes, the same participants. Subgroups were randomly recruited from the vitamin C and E group and the placebo group, as indicated in Figure 1 and explained under “Acute experiment” (line 191-193).
Our idea with placing the acute experiment midway in the intervention was to get a mechanistic understanding of the potential prolonged effects. You are correct in that we may not be able to differentiate between acute and prolonged supplementation; however, the focus is on how vitamin C and E may affect adaptation to strength training over time, so it is not critical that potential effects are due to the supplementation before/after the exercise session and/or due to weeks of supplementation before this experiment. However, the responses to the exercise session will be expressed as relative changes, in order to reduce the potential impact of prolonged supplementation. Tests midway, before the acute experiment may be used to control for effects of the supplementation before the acute experiment.

To address this, we have added a short paragraph under “Limitations” in the Discussion (line 556-562).

Reviewer #2

Authors: We would like to thank this reviewer for thoughtful and fair critique and suggestions. Your recommendations have improved the manuscript.

An interesting study with several parameters to be assessed. There is a sound introduction and hypothesis and the methods section is pretty easy to follow. However, there are several aspects that need to be addressed so the study is easier to be followed by the reader.

Since women were included in the study and a few things need to be clarified. What time of their menstrual cycle was data collected? Did any woman take contraceptives?

Authors: This is a fair critique. We are aware of the potential effects of menstrual cycle and contraceptives (5104). Unfortunately we were not able to control for the menstrual cycle of the woman. This is now stated under “Participants” (line 129-130). About 1/3 of the women used contraceptives, but they were equally distributed among the two groups. Furthermore, others have found similar responses to strength training in contraceptive users and non-contraceptive users (5102). Thus, we acknowledge this is a potential confounding factor in the study, but we believe that this fact will have minor effects on the main outcomes. Nevertheless, we will do statistics and control for sex. Further analyses may be done to determine potential impact of contraceptive use. Thank you for making us aware of this issue.

Why were there different apparatus (DXA) to assess body composition in the two groups?

Authors: Simply because the experiments on young and elderly participants were conducted in geographically different places (line 166-167), and the available machines were different. As comparisons on absolute values only will be done within each experiment, we believe that this is no problem.

What is the value of adding Bio-impedance to assess body composition since
DXA was used?

Authors: We wanted to add some midway time points, and chose to use the bio-impedance for this. The reason was partly to reduce the total radiation dose and partly because of convenience.

There are several tests that were conducted either on young or old individuals alone. An explanation needs to be given behind the reason of this selective approach.

Authors: As we found it most interesting to study trained young participants and untrained elderly participants, we had no intention to compare the young and the elderly – the training regimes had to be quite different, as mentioned under Limitation in the Discussion. Moreover, the two studies were done in geographically different places, which restricted us to use equipment/measurements that were available on each site. E.g., MRI and MVC were not possible to include in the study with the elderly participants. Finally, certain tests were more interesting for the particular age group.

Line 376: It is not clear how many muscle biopsies in total were performed on young individuals.

Authors: Thank you for pointing this out. Each young participant had two biopsies sampled from the right leg (one before and one after the 10 week intervention period). The participants that also took part in the acute experiment had four additional biopsies sampled from the left leg. This is now better explained under “Muscle tissue sampling and analyses” (line 384-387).

Ln 397 Why is glutathione measured in plasma and not in red blood lysate?

Authors: Glutathione is indeed planned to be analyzed in both plasma and red blood cells, as now stated under “Blood sampling and analyses” (line 417-418).

Ln 401: At what time point of the whole experiment the procedure to assess protein synthesis is taking place?

Authors: 4-6 week into the training intervention period. This is stated under “Acute experiment” (line 192) and in Figure 2.

Ln 401: Indicate how much and how many blood samples were taken during that procedure.

Authors: 11 samples and a total for 22 ml. This is now better explained under “Protein synthesis” (line 431) and in Figure 3.

Ln 477-479: Please re-phrase.

Authors: The sentence is re-prehased (line 502-504).
Ln 505-506: Please re-phrase.

Authors: The sentence is re-prehased (line 527-529).

Ln 559 Replace “antioxidants” with “antioxidant”.

Authors: Done. Thank you.