Author's response to reviews

Title: Effect of beetroot juice on lowering blood pressure in free-living, disease-free adults: a randomized, placebo-controlled trial

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Author's response to reviews: see over

We thank the reviewers for their helpful comments, which we have carefully considered in preparing this revised manuscript. Please find our detailed responses below:

Reviewer: Tanja Sobko

Major Compulsory Revisions

Congratulations on a nicely designed study. This is an important trial as it reports the impact of beetroot juice on BP. The strength of this study is that it was randomized, crossover as well as the BJ was given a part of an ordinary diet.

The drawback is that the trial individuals, half of the women, have been using pharmaca, which I believe might influence the effect of the treatment.

A discussion on medication has now been included (lines 181 – 186):

"The crossover design of the study should have eliminated any individual variation in blood pressure due to any medication taken daily on the two 24 h periods that BP was measured. However, the possibility of the effect of medication or diet (e.g. sodium intake) on the outcomes of the trial cannot be discounted and ideally the experiment should be repeated in non-medicated individuals and the dietary intake recorded."

More importantly, the authors should take into consideration the BMI of the subjects, as it seems that the studied group are overweight subjects. In order to connect this effect with nitrate, abundant in BJ, blood samples should be taken and analyzed. In my opinion, the issues above should be handled or at least discussed.

Text has been added regarding the BMI of the subjects and the benefit of testing NO_3 in blood samples (lines 252 – 259 and 269 - 270):

"Using an even larger group of volunteers, with different characteristics (e.g. on average, lower / higher BMI or age) would be further beneficial, as the group studied here were on average middle-aged and slightly overweight. Although it was not undertaken in the present study, it would also be preferable to measure plasma NO_3 levels before and after consumption of the BJ and PL in order to establish a causative relationship between the NO_3 present in beetroot and the effect on plasma NO_3 levels leading to a reduction in BP. This would assist in eliminating other possible causes of the effect seen, such as the diuretic property of beetroot."

"Additional studies with beetroot and apple juice in larger groups of free-living men and women are needed to fully assess the efficacy such a dietary intervention at a public health level in the treatment of cardiovascular disease and to determine the exact mechanism of action."

Discretionary Revisions

It would be interesting to see the table 3 in a figure form, to have a more illustrative view over the 24 hrs effect on BP.

We feel that more useful information is obtained from presenting the data in tabular form in this instance.

Reviewer: Decio Armanini
The authors have measured blood pressure before and after intake of a beverage containing both Beetroot and Apple juice. Overall, there was no significant difference on the two treatments was found except daytime pulse pressure.

In the planned Ancova in men only BJ treatment was significantly different from placebo. The difference was not evident in women but in the table 2 it is reported that 8 subjects were treated and in particular 5 were taking hormonal contraception. It is well known than this treatment can affect blood pressure. Beetroot is a natural diuretic and the explanation of the results could be related to this effect. To demonstrate that the effect is due to NO3 the authors should have measured the plasma NO3 before and after the consumption. No indication is given of the sodium intake. The authors do not report data on the possible cause for the increase of blood pressure. Both groups were consuming apple juice and another explanation for the lack of differences between patients and controls could be due to some opposite effect of apple juice. In conclusion the study should compare pure beetroot extract versus placebo, measuring the serum NO3 content before and after administration.

We have now mentioned other possible causes of the effect seen, including sodium intake, apple juice consumption and medication (lines 181 – 186 and 239 - 244):

"The BJ and PL used here contained 28% apple juice. It is plausible that the apple juice may have had some contrary effect on BP, thus partially negating or cancelling the effect of the beetroot on lowering BP. While the literature actually supports a BP lowering effect of quercetin (found in large amounts in apples) [24], the experiment could be repeated using pure beetroot juice to eliminate any possible effect of other components present in the BJ."

We agree that it would be preferable to measure NO_3 as suggested by the reviewers and have made note of this in the text (lines 255 – 259 and 269 - 270):

"Although it was not undertaken in the present study, it would also be preferable to measure plasma NO_3 levels before and after consumption of the BJ and PL in order to establish a causative relationship between the NO_3 present in beetroot and the effect on plasma NO_3 levels leading to a reduction in BP. This would assist in eliminating other possible causes of the effect seen, such as the diuretic property of beetroot."

"Additional studies with beetroot and apple juice in larger groups of free-living men and women are needed to fully assess the efficacy such a dietary intervention at a public health level in the treatment of cardiovascular disease and to determine the exact mechanism of action."