Author's response to reviews

Title: Short-term effects of nicotine-free e-cigarette compared to traditional cigarette in smokers and non smokers

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Author's response to reviews: see over
Dear Editor,

first of all we thank you and the reviewers for your careful reading and useful comments. We have hopefully replied to all the comments raised by the two referees and we really hope that you will find the revised version of the manuscript suitable for publication.

Concerning your specific comments, here you find a point-by-point answers:

1. **Name of Ethics Committee:**

   Please update your ethics statement to include the name of the ethics committee that approved your study.

   We have added now

2. **Acknowledgements:**

   We do NOT have to acknowledge anyone who contributed towards the article by making substantial contributions to conception, design, acquisition of data, or analysis and interpretation of data, or who was involved in drafting the manuscript or revising it critically for important intellectual content, but who does not meet the criteria for authorship

3. **Copyedit:**

   We recommend that you ask a native English speaking colleague to help you copyedit the paper.

   We have asked a mother tongue consultant to check the English of the manuscript

4. **Track Changes:**

   Please also highlight (with 'tracked changes'/coloured/highlighted text) all changes made

   We have done so

Once more thank you for giving us the possibility to revise the manuscript and hopefully to improve it

Sincerely yours,

Stefano Nava

Dear reviewer #1,

first of all we thank you for your careful reading and useful comments

Here you find a point-by-point answers to your comments

Major compulsory revisions

1. **The reason for which it was chosen that specific NFe-cig for this study should be clearly stated. There are tens of different electronic cigarettes currently available on the market. Throughout the manuscript, it should be specified that the observed results are related to that specific NFe-cig evaluated in this study, and cannot be generalized. For example: Discussion, page 9, line 180 “In this study we have demonstrated that the use of NF e-cigarettes was not associated with major acute physiological changes”; Conclusions, page 12, line 271 “In conclusions in this randomized trial we have shown that the use NF e-cigarettes was found to have no immediate adverse physiologic effects after short-term use”**.
We thank once more this reviewer for pointing out this important problem. There was no specific reason because we have chosen this e-cigarette. Our internet search was based on NFe-cigarettes available and produced in our Country (i.e. Dea-Flatech-Flavour Roma etc) and we finally decided to use the Angelica liquid mainly because it was based in our city (Bologna) and probably easier to buy. We have changed, as suggested, all the “generalization” about it in the whole text.

2. The effects assessed in the present study are not all “physiological”. For example, it is not “physiological” for a non-smoker being exposed to high levels of CO. Moreover, this is not a “randomized trial”. Randomization was correctly applied when testing subjects. However, the study subjects are a convenient sample selected among local health personnel (“pulmonary fellows or attending physicians”, page 4 line 50). The terms “physiological effects” and “randomized trial” should be modified in the title and throughout the manuscript, including the keywords

We agree with these precious comments and we have changed, accordingly

3. The study was conducted on a very small sample of subjects. This is an important limit of the study and should be adequately discussed, also in terms of statistical analyses and obtained significant results.

We reviewer is right. We forgot to insert in our statistical analysis the sample size calculation, that allowed us to decide the number of subjects to be enrol. We have now added both this calculation, and the limitation of a small sample size in the appropriate sections. In particular: Prokhorov et al. found a decrease of 2.14% of predicted value of FEV$_1$ in 18 regular smoker volunteers after smoking one traditional cigarette [4]. Since no value of the standard deviation of the within-subject difference was reported, we have hypothesized this value as 3.26% (one third of the value reported as overall standard deviation in the Prokhorov et al. study; i.e., 9.78%) [4]. By comparing these values versus no effect of the e-cigarette, we had to study 20 subjects in order to be able to reject the null hypothesis with probability (power) equal to 0.80 and a two-sided type I error probability of 0.05. Thus the sample size was fixed to 20 subjects (10 smokers and 10 nonsmokers) by hypothesizing similar effect of smoking one traditional cigarette between smokers and nonsmokers. The sample size was estimated by means of the “PS Power and Sample Size Calculations” software.

4. There are evidences that electronic cigarettes may cause adverse effects such as cough, throat irritation, nausea etc. In the present study there is no mention of the possible subjective symptoms that the study subjects may have experienced. It should be noted that non(never)-smokers were enrolled in the study, that is subjects that potentially could have important acute symptoms when exposed to acute active electronic/tobacco smoking for the first time. This limit of the study should be clearly discussed.

We have now reported in the Results of the “acute” symptoms experienced by the non-smokers (indeed very mild !).

5. The authors seem to attribute the adverse effects of the “electronic cigarettes” to their content in nicotine (see Discussion, page 9, lines 196-199). However, this is a speculative statement, since (at least to the best knowledge of this reviewer) there is no evidence with regard to this issue.
Studies supporting this affirmation should be quoted otherwise this part of the discussion should be modified or eliminated. Agree with the reviewer and therefore we have eliminated this sentence.

6. The authors seem to attribute the decrease in FEV1 and FEF25, observed in smokers when using the NFe-cig, to some component different from nicotine (see Discussion page 11, lines 246-252). Also in this case, studies supporting this affirmation should be quoted. Moreover, the higher levels of FeCO observed in smokers, as compared to non-smokers, may be explained by the previous CO exposure from tobacco smoking in the formers. Indeed, according to study protocol, smokers should refrain from smoking in the 6 hours preceding the experimental testing while the half-life of expired CO is about 4 hours, depending on exercise.

We are sorry because we did not quote our statement about the potential mechanisms leading to the decrease in FEV1 and FEF25. As a matter of fact, you are right, there are NO studies on the topic. We have added it to the Discussion. Agree also on the fact that the higher levels of FeCO in smokers may be related to previous CO exposure. We have added that.

7. Discussion, page 10, lines 220-225. It is not immediately clear to this reviewer how a “repetitive acute smoke effects may cumulate and ultimately lead to irreversible damage”. Possible long-term effects due to “electronic smoking” should be assessed in specifically designed studies and cannot be foreseen on the basis of the short-term effects observed in the present study, based on a single exposure of 5 minutes.

Yes, we agree with the reviewer that this comment was a bit “strong” and therefore we have erased it.

8. Methods, page 4, line 63. “All subjects were asked to use a similar pattern and frequency of smoke aspiration”. This is a very surprising instruction for the study protocol, since it seems difficult that a non-smoker may smoke a tobacco cigarette for 5 minutes, probably for his/her first time, as a regular smoker.

Right. These were the “theoretical” instructions given to the subjects and it is not granted that they may fully simulated the smokers habit. We have now better explain the concept.

Minor essential revisions
9. Replace “pack/year” with “pack-years” throughout the manuscript
10. Results, page 8 lines 154 and 156. Replace “no smoker subjects” / “no smokers” with “non smokers”
11. Discussion, page 9, line 187 “Among the other measures to prevent tobacco smoking and to quit smoking”. According to the current available scientific evidences and guidelines for smoking cessation, the electronic cigarettes are not a preventive or treatment measure for tobacco smoking.
12. Discussion, page 9, line 199. Correct the misprint “of the of the”.
13. Discussion, page 11, line 237. Replace “that in smokers” with “than in smokers”.
14. Discussion, page 11, line 238. Replace “have induce” with “have induced”.
15. Discussion, page 11, line 240. Replace “that reported” with “than that reported”
We have changed everything, accordingly

Dear reviewer #2,

first of all we thank you for your careful reading and useful comments
Here you find a point-by-point answers to your comments

**Abstract. This would be improved by adding the data and not just quoting p values**
We have done so.

**Background This is not well written. It needs more fluency in the statements so that the points logically lead from one to the next.**
We have now extensively re-written this part of the manuscript, and hopefully now it will full fill your requirements

**Methods**
*Please note this is not a ‘trial’ – change to ‘study ’ throughout.*
We have done so

**Subject characteristics are usually reported in the Results. Please move**
We have done so

**Protocol/Design Ad Lib use of cigarettes: this is unfortunate and suggests that there was no standardisation between subjects: it is likely that a current smoker would smoke more than a non – smoker. Please address this point in the Discussion at least**
We thank once more the reviewer for highlighting this important comment. We have now insert the issue in the Limitations section

**P5 line 99 is that paragraph referring to the FENO, Co or the spirometry?**
It refers to ..... This has been hopefully clarified

**Discussion This is poorly organised. The paragraph starting at line 216 needs to carry on after the para starting at line 196.**
We now re-organized this part and we hope that it may be more clear

**The effectiveness of this device as an aid to smoking cessation can be kept, although it is not really relevant to this paper and could be made much shorter**
We agree and we have now significantly shortened this part

**As noted about more needs to be done to discuss the limitations of the study design: only crude spirometry has been assessed, DLCO and lung volumes could have been performed and perhaps measurements of airway reactivity and particulate/vapour burden.**
As for the “ad lib use” comment, we have now focused more the limitations of the study

**Abstract – change ‘claimed to have been expressed’**
*Line 127: remove ‘behaviour’ and change to ‘The FECO values ,,,are shown in Figure 1’*
*43 161, not significantly different*
*146, 148 substitute’ changes’ for ‘modifications’*
135, 148 Change to subjects studied rather than studied subjects
150 Change 'homogeneous' to 'similar'
152 effect of smoking the two cigarette types..
154, 156 non-smoking subjects
182 induced
216 concerns

We have now changed all these typos. Thanks once more!