

Reviewer 1

1. Line 33 The reference denoted should be a square bracket based on MDPI guidelines

We have reformatted the references in this revised manuscript to conform with the MDPI guidelines

2. Line 61 is this correct including “11-dh-thromboxane B₂”

We have provided the full name for this metabolite, 11-dehydro thromboxane B₂.

3. Line 65 What are healthy smokers here?

Broadly, “generally healthy smokers” do not exhibit uncontrolled clinical symptoms of disease or sickness. We have included some key inclusion and exclusion criteria in the Supplementary section.

For example, we have excluded smokers with the following conditions:

- Presence of clinically significant uncontrolled cardiovascular, pulmonary, renal, hepatic, endocrine, gastrointestinal, psychiatric, hematological, neurological disease, or any other concurrent disease or medical condition that, in the opinion of the Investigator, makes the study participant unsuitable to participate in this clinical study.
- History, presence of, or clinical laboratory test results indicating diabetes.
- Systolic blood pressure of > 160 mmHg or a diastolic blood pressure of > 95 mmHg, measured after being seated for five minutes.

4. Line 69-70 Similar examples need to understand the Biomarker of exposure

We have now added examples of biomarkers of exposure in the manuscript.

“Biomarkers of exposure include nicotine, carbon monoxide and tobacco specific nitrosamines (NNAL, NNN, NAT and NAB). Exposure to nicotine is measured as a composite of nicotine and five other metabolites and is expressed as total nicotine equivalents. “

5. Line 86 Why only two weeks? What would be effects after one month of abstinence.

Our objective was to identify mechanistic BoPH that rapidly respond to smoking cessation. To ensure that smokers comply with study procedures (i.e., abstinence) we used a clinical confinement design for this study. Extended confinement presents numerous challenges; for example, subject recruitment and retention are among them. Based on findings from our cross-sectional ([Prasad et al 2016](#) and others cited in the manuscript) and previous 5- and 7-day confinement studies ([Makena et al 2019](#) and [Milly et al 2022](#)), we designed this two-week confinement study for the evaluation of biomarkers in smoking abstinence.

The declines in biomarkers of exposure in smoking abstinence are dependent on exposure and their half-lives. As shown in Table 2, many of the biomarkers of exposure have reached near-maximal decline following two weeks of smoking cessation, and biomarkers such as NNAL are expected to further decline with continued abstinence to baseline levels found in non-smokers.

Generally, changes in Biomarkers of potential harm will be dependent on the impact of smoking on specific pathways/biomarkers. For LTE₄ and 2,3-d-TXB₂, declines in these two biomarkers were approaching the levels found in non-smokers/tobacco users. Following extended smoking cessation, the levels of these biomarkers could reach and stabilize at the levels found in non-smokers in the general population.

6. Line 214-215 Please explore the sentence for clarity

We have simplified the sentence per Reviewer's suggestion.

"We demonstrated that LTE₄ (a marker of airway hypersensitivity) and 2,3-d-TXB₂ (a marker of platelet aggregation) rapidly declined in generally healthy smokers in response to smoking abstinence."

7. Line 242 How the authors describe these result with 14 days

The current study and the one by [McElroy et al 2019](#) differ in several ways, including in subject demographics. For example, our study enrolled 70 subjects in two age groups (24-34 years and 35-60 years), whereas McElroy et al recruited 15 subjects with a mean age of 44.9 years, ranging from 18-65 years, and followed the subjects for nearly 3 months of smoking cessation. They concluded that declines in 8-iso-PGF₂ α are modest (27%) and occur slowly upon smoking cessation. We found that the decline in 8-iso-PGF₂ α in the 24-34 years age group at 14 days was 29%, comparable to that reported by McElroy et al; however, we did not find any statistically significant changes in the 35-60 years age group. It is possible that longer and sustained smoking abstinence may be required to detect a more uniform and steady decline across different study populations.

8. Line 257 needs more references to add up. "Many studies have reported that"

We have rephrased the sentence and added additional references.

"Several studies have reported that levels of 11-dh-TXB₂ are also higher in smokers compared to non-smokers (including moist snuff users) [12-14]."

9. Line 301 What does it indicate please describe some introductory lines

Per the reviewer's suggestion, we have added context/introduction to changes in the levels of FeNO in smoking cessation.

“Oxidative stress caused by cigarette smoking has been known to reduce the levels of FeNO in smokers’ lungs [1], and the levels of exhaled nitric oxide are reported to increase following smoking cessation [39,40].”

10. All references formats should be modified based on MDPI guidelines

The references are now formatted using an *EndNote* template file for the MDPI Chicago Style, available on the [MDPI website](#).