PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Protocol to Assess the Impact of Tobacco-Induced Volatile Organic Compounds on Cardiovascular Risk in a Cross-Sectional Cohort: Cardiovascular Injury Due to Tobacco Study
AUTHORS	Keith, Rachel; Fetterman, Jessica; Riggs, Daniel; O'Toole, Timothy; Nystoriak, Jessica; Holbrook, Monika; Lorkiewicz, Pawel; Bhatnagar, Aruni; DeFilippis, Andrew; Hamburg, Naomi

VERSION 1 – REVIEW

REVIEWER	Dr. Markos Klonizakis Centre for Sport and Exercise Science, Sheffield Hallam University, Sheffield, U.K.
REVIEW RETURNED	03-Nov-2017

GENERAL COMMENTS	This is an interesting and well-thought study. Some things to consider include: a) Why didn't you exclude people with a history of micro- or macro-vascular disease? Surely this would affect your findings. b) How did you come up with the required number of participants? I'm surprised not to see a formal sample size calculation for a study that is bound to be so costly. c) How much time will each session take for the participants? The study might be already under-way but it will be interesting to see what is the patient burden and if there is any compensation/reimbursement for the participants. d) Will you exclude purposefully participants in order to achieve a balance between Caucasian and African American participants? e) The participants' journey should become more clear, from the beginning until the end. f) As this is a study protocol, you should also provide information on the equipment used and also state the lab's reproducibility rate - especially for FMD assessments (where precision and a high reproducibility are of major importance). and finally, g) is the study registered in one of the trial databases? If not, it should be.

REVIEWER	Elizabeth Barksdale Boyle National Academies of Sciences, Engineering, and Medicine
REVIEW RETURNED	02-Dec-2017

GENERAL COMMENTS	This is a paper which is documenting the protocols being used in the Cardiovascular Injury due to Tobacco Use study. This paper does

	not present result of the study or evaluate the research question, relating the early cardiovascular endpoints to tobacco biomarkers. The introduction and abstract should be rewritten to clarify that this is a paper documenting methodology and not a scientific evaluation of hypotheses.

VERSION 1 – AUTHOR RESPONSE

December 13, 2017
Emma Gray
Assistant Editor
BMJ Open

RE: “Assessing the Impact of Tobacco-Induced Volatile Organic Compounds on Cardiovascular Risk in a Cross-Sectional Cohort: Cardiovascular Injury Due to Tobacco Study”

Dear Ms. Gray,
Thank you for an opportunity to revise manuscript ID bmjopen-2017-019850 entitled "Assessing the Impact of Tobacco-Induced Volatile Organic Compounds on Cardiovascular Risk in a Cross-Sectional Cohort: Cardiovascular Injury Due to Tobacco Study". The comments from the Editors and reviewers were insightful and led to multiple revisions of this manuscript. To address the valid concern of clarifying that this is a protocol paper we have changed the title to “Protocol to Assess the Impact of Tobacco-Induced Volatile Organic Compounds on Cardiovascular Risk in a Cross-Sectional Cohort: Cardiovascular Injury Due to Tobacco Study” and updated the abstract and introduction. Additionally we have revised the paper to include more information about measurement methods and the participant’s journey.
Please find a point by point response to the comments provided below.
We are happy to provide additional revisions or explanation if necessary.
Sincerely,
Rachel Keith, PhD, APRN
Assistant Professor
University of Louisville School of Medicine

Editor Comments to Author:
- Please make it clear in the title that this is a protocol paper.
To address this we have changed the title of the manuscript to “Protocol to Assess the Impact of Tobacco-Induced Volatile Organic Compounds on Cardiovascular Risk in a Cross-Sectional Cohort: Cardiovascular Injury Due to Tobacco Study”. (Page 1)

Reviewer(s)' Comments to Author:
Reviewer: 1
a) Why didn't you exclude people with a history of micro- or macro-vascular disease? Surely this would affect your findings.
Our study design is to enroll only healthy individuals, so we do exclude individuals with known micro- or macro-vascular disease. Our exclusion criteria includes: diabetes, hypertension, renal insufficiencies and clinically established cardiovascular disease (including myocardial infarction, angina, heart failure, peripheral vascular disease or stroke). To clarify the exclusion process for the manuscript we added text to the exclusion criteria that mentions known cardiovascular disease (see page 6 ln 124-127). In addition, we measure creatinine and ankle brachial index. If necessary we can further refine our population or do sensitivity analysis for individuals who are outside of a normal range.

b) How did you come up with the required number of participants? I'm surprised not to see a formal sample size calculation for a study that is bound to be so costly.
Formal sample size calculations were performed prior to study implementation. We report on the sample size estimates on page 15. We calculated the sample size needed to determine a clinically relevant difference in flow-mediated dilation. We planned to enroll a larger sample size than would be required for FMD alone in order to account for multiple endpoints tested as well as to evaluate correlations with the volatile organic compounds we plan to measure. This information was added to lines 271-273 on page 15.
c) How much time will each session take for the participants? The study might be already under-way but it will be interesting to see what is the patient burden and if there is any compensation/reimbursement for the participants.
Thank you for the suggestion to include this information. As added on page 7-8 lns 153-156 study visits take 90 minutes and participants were compensated appropriately for their time.
d) Will you exclude purposefully participants in order to achieve a balance between Caucasian and African American participants?
Our enrollment procedures seek to enroll participants of multiple racial and ethnic groups given the prevalence of tobacco use. We do not plan to exclude participants to balance the groups. We will evaluate for effect modification by race in the analysis.
e) The participants' journey should become more clear, from the beginning until the end.
To clarify the participant's journey we have added a flow chart (Figure 2) describing the experience on page 8 under the section for overall study procedure.
f) As this is a study protocol, you should also provide information on the equipment used and also state the lab's reproducibility rate - especially for FMD assessments (where precision and a high reproducibility are of major importance).
Per reviewers suggestion we have included information on our protocol for FMD (page 12-13 lns 220-230). We have previously reported reproducibility with intra- and inter-observer correlation coefficients of 0.98 to 0.99 for brachial diameter and 0.78 to 0.92 for FMD.
g) is the study registered in one of the trial databases? If not, it should be.
Though the researchers appreciate the benefit of registering trial, per guidelines on clinicaltrials.gov, as an observational study, not an interventional study, so never registered.

Reviewer: 2
This is a paper which is documenting the protocols being used in the Cardiovascular Injury due to Tobacco Use study. This paper does not present result of the study or evaluate the research question, relating the early cardiovascular endpoints to tobacco biomarkers. The introduction and abstract should be rewritten to clarify that this is a paper documenting methodology and not a scientific evaluation of hypotheses.
Thank you for this suggestion. To clarify that this paper is a protocol paper we first added a statement to the title. Secondly the abstract methods section was updated to read "We present the design and methodology of the CITU study a cross-section observational tobacco study…" (pg2 ln 41-42) and took out a potential future completion date for the study (pg 2 ln 43). Lastly in the introduction we state "Thus, in this paper we present the design and methodology of the Cardiovascular Injury due To Tobacco Use (CITU) study …." (pg 5 ln 99-100)

VERSION 2 – REVIEW

REVIEWER	Elizabeth Boyle National Academy of Sciences
REVIEW RETURNED	23-Jan-2018

GENERAL COMMENTS	I'm not sure if the authors saw my comments which were submitted

	as an attachment. I'm noting this because I did not see a response to my comments in the letter. However, it looks like most of them were addressed in these revisions. The first comment, that I don't think was addressed in the revisions is, that the VOC metabolites they will measure have limitations, short half-lives, and some are not very specific to the parent compound, for example, TTCA is not specific to carbon disulfide exposure as it is also associated with the consumption of brassica vegetables. It might not be necessary to discuss all these metabolites, but it would be worth mentioning and planning for the inherent issues with the primary ones in the analysis. Second, the single spot urine sample is not an adequate exposure measure for these metabolites. They intraindividual variability is high for these metabolites. If more than one urine sample is going to be collected from a participant it should be clearly stated. Third, the statistical methods section does not actually describe the tests they propose to use to measure the research question of interest. I would expect to see a discussion of models to relate measures of cardiovascular disease injury with VOC exposure. What the authors' refer to as dose dependence, i.e., the association between the biomarkers and smoking as reported by questionnaire. This approach seems reasonable. However, the cited Benowitz article shows that biomarkers of tobacco are only modestly correlated with the number of cigarettes per day. Moreover, the statement that the US population smokes between 15-20 cigarettes per day is untrue. The Benowitz article only enrolled smokers (128) and the mean CPD in that population was between 10 to 20. I would also reorder this section since the sample size calculations are completed.

VERSION 2 – AUTHOR RESPONSE

February 14, 2018
Emma Gray
Assistant Editor
BMJ Open

RE: “Assessing the Impact of Tobacco-Induced Volatile Organic Compounds on Cardiovascular Risk in a Cross-Sectional Cohort: Cardiovascular Injury Due to Tobacco Study”

Dear Ms. Gray,
Thank you for a second opportunity to revise manuscript ID bmjopen-2017-019850 entitled "Assessing the Impact of Tobacco-Induced Volatile Organic Compounds on Cardiovascular Risk in a Cross-Sectional Cohort: Cardiovascular Injury Due to Tobacco Study". We apologize for missing a reviewrs comments the first time around and have made the appropriate revisions of this manuscript. Please find a point by point response to the comments provided below.
We are happy to continue to work with editorial staff as needed for this manuscript.
Sincerely,
Rachel Keith, PhD, APRN
Assistant Professor
University of Louisville School of Medicine

Reviewer(s)' Comments to Author:

Reviewer: 2

I'm not sure if the authors saw my comments which were submitted as an attachment. I'm noting this because I did not see a response to my comments in the letter. However, it looks like most of them were addressed in these revisions.

Initially we did not see your comments and hope we address them adequately below.

- The first comment, that I don't think was addressed in the revisions is, that the VOC metabolites they will measure have limitations, short half-lives, and some are not very specific to the parent compound, for example, TTCA is not specific to carbon disulfide exposure as it is also associated with the consumption of brassica vegetables. It might not be necessary to discuss all these metabolites, but it would be worth mentioning and planning for the inherent issues with the primary ones in the analysis.

We thank the reviewer for this comment and believe it is an important point. WE understand that there are multiple sources of exposure to VOCs and hope to draw reader's attention to this with our manuscript revisions. We do believe that as demonstrated by Jain et al., levels in smokers exceeds that of the general population and there are multiple relevant metabolites to consider. We did add a section to the analysis plan to help clarify how we may address this for either single pollutants or part of a multipollutant model. Specifically the text states:

"Many VOC metabolites have relatively short half-lives that range from 2 - 25.2h, 30 31 but given the constant pattern of tobacco product use by most users, spot collection reflects recurrent use. Moreover, even though some VOC metabolites, such as HPMA, are known vary with time of day,29 synchronizing the study visits and requiring a tobacco fast is likely to minimize diurnal variations in metabolism. Our past work has shown that spot-urine collected at the same time of day reliably reflects daily VOC exposure and is correlated to CVD risk32. (page 8)

Humans are exposed to VOCs from a variety of sources including indoor and outdoor environments as well as diet. The most significant sources of ambient exposure ambient are air pollution, car exhaust, household products, personal hygiene products, and solvents26 27. Although concurrent exposures from multiple sources could confound attribution to smoking, the levels of urinary metabolites of these VOCs in smokers far exceeds those measured in non-smokers exposed to typical sources of VOCs 28. (page 8)

- Second, the single spot urine sample is not an adequate exposure measure for these metabolites. They intraindividual variability is high for these metabolites. If more than one urine sample is going to be collected from a participant it should be clearly stated.

Our previous work, and that of others, suggests that a single spot urine is adequate for VOC analysis. Therefore, we are only collecting urine at one time point. In an attempt to standardize time since last exposure, which can effect VOC metabolite levels, we request a standard tobacco and food fast. Additionally we schedule all appointments at similar times of day to account for diurnal variations in metabolism of VOCs. Accordingly, we have edited the section that discusses urine collection to read:

"Standard clean catch urine specimens are obtained from participants. Though only a single urine time point is collected, previous studies show spot urine measurements correlate well with 24-hour urine collections29. Many VOC metabolites have relatively short half-lives that range from 2 - 25.2h, 30 31 but given the constant pattern of tobacco product use by most users, spot collection reflects recurrent use. Moreover, even though some VOC metabolites, such as HPMA, are known vary with time of day,29 synchronizing the study visits and requiring a tobacco fast is likely to minimize diurnal variations in metabolism. Our past work has shown that spot-urine collected at the same time of day reliably reflects daily VOC exposure and is correlated to CVD risk32."

- Third, the statistical methods section does not actually describe the tests they propose to use to measure the research question of interest. I would expect to see a discussion of models to relate measures of cardiovascular disease injury with VOC exposure. What the authors' refer to as dose

dependence, i.e., the association between the biomarkers and smoking as reported by questionnaire. This approach seems reasonable. However, the cited Benowitz article shows that biomarkers of tobacco are only modestly correlated with the number of cigarettes per day. Moreover, the statement that the US population smokes between 15-20 cigarettes per day is untrue. The Benowitz article only enrolled smokers (128) and the mean CPD in that population was between 10 to 20. I would also reorder this section since the sample size calculations are completed.

To clarify our analysis plan we have included more details as follows. Additionally we correct the typical cigarettes per day to read 10-20 to match the Benowitz citation.

"Differences in VOC's between product groups will be tested using ANOVA for normally distributed data or Kruskal-Wallis test for non-normal data. The association between primary outcomes of vascular function as well as circulating markers of cardiovascular injury with individual VOC levels will be analyzed using multiple regression models, adjusting for appropriate confounders. Additionally, because we have multiple VOC's, which are highly correlated, we will use methods such as LASSO to identify the VOC's that are most associated with the outcomes of interest. Multipollutant approaches, such as principal component analysis (PCA), will be used to test whether overall VOC exposure is associated with the health outcomes. (page 15)"

VERSION 3 – REVIEW

REVIEWER	Elizabeth Boyle National Academies of Sciences, Engineering, and Medicine
REVIEW RETURNED	18-Feb-2018

GENERAL COMMENTS	The authors did a great job revising the manuscript.