PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Short-Term Abstinence from Alcohol and Changes in Cardiovascular Risk Factors, Liver Function Tests and Cancer-Related Growth Factors: A Prospective Observational Study
AUTHORS	Mehta, Gautam; Macdonald, Stewart; Cronberg, Alexandra; Rosselli, Matteo; Khera-Butler, Tanya; Sumpter, Colin; Al-Khatib, Safa; Jain, Anjly; Maurice, James; Charalambous, Christos; Gander, Amir; Ju, Cynthia; Hakan, Talay; Sherwood, Roy; Nair, Devaki; Jalan, Rajiv; Moore, Kevin

VERSION 1 – REVIEW

REVIEWER	Vasilios G. ATHYROS, MD Aristotle University, Thessaloniki, 55132, Greece
REVIEW RETURNED	07-Dec-2017

GENERAL COMMENTS	Please leave your comments for the authors below The study bmjopen-2017-020673 by Gautam Mehta et al, entitled “The Effect of Alcohol Consumption on Cardiovascular Risk Factors, Liver Function Tests and Cancer-Related Growth Factors” has relevance to the audience of Journal of BMJ OPEN. The aim of this paper was to assess the effects of abstinence from alcohol on metabolic risk factors and cancer-related growth factors. Authors conclude that abstinence from alcohol in moderate-heavy drinkers improves insulin resistance, weight, blood pressure and cancer related growth factors. Major Comments for the authors 1. This is a confirmation prospective (one month bool and other measurements and 6-8 months telephone questionnaire) 2. The number of authors is too great (17) for the number of participants included (97 drinkers and 48 controls, those that continued alcohol consumption).Thus there was no randomization and patients were divided in groups according to their statement that they will or will not keep on drinking). Personally, I do not know if it is very ethical not to do everything to persuade the participants to abstain from drinking and use them as a control group. 3. The authors report that the participants come from a single center (probably the Institute for Liver and Digestive Health, Division of Medicine, Royal Free Campus, Rowland Hill Street, London NW3 2PF, UK), but the authors come from 9 clinics, including an

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	anesthesiology clinic from USA (12. McGovern Medical School, University of Texas Health Science Center at Houston, Texas 77030, USA). There is disequilibrium here. 4. “Participants were assessed at baseline and after one month”, thus the presented results represent changes that happened in one month of abstinence. The authors should include in the title the words “short time”. 5. Are there any practical implications of the study results?

REVIEWER	Tim Stockwell Canadian Institute for Substance Use Research University of Victoria Canada
REVIEW RETURNED	22-Dec-2017

GENERAL COMMENTS	I like this paper a lot. I think it contributes well to an emerging literature about the potential benefits of temporary abstinence from alcohol. I have a small number of general observations and a few specific suggestions on small issues that can be tidied up and I think improve the final product a little. General comments: 1. As you acknowledge, there was no randomisation. However, it is hard to think of a way in which intention to abstain or not abstain in itself could inadvertently lead to significant changes in the various biomarkers used. I think this is a good, pragmatic and convincing design to test your hypotheses. 2. I would have liked more information about the sample and exactly how they were recruited. You state that it was by email. It seems unusual to be able to locate so many people planning to give up alcohol for a month. How was the invitation to participate worded? Did you ask people to volunteer who were planning to abstain or, alternatively, to continue drinking? Was recruitment related to a Dry January or similar campaign? Were participants paid for their time? 3. I think your findings re insulin resistance and drinking are particularly interesting. 4. Another strength of the study you might stress is the very high follow-up rate. This seems highly unusual - almost unbelievable! I assume you had a captive population or paid them a lot? I would have liked more details to get a sense of how that was achieved. Specific points P4 line 7: You say alcohol is the 3rd largest risk factor in the GBD estimates but you cite a 2009 paper. The rank of alcohol has jumped around a lot in recent years with more recent estimates and their updates. Please check for the latest - last time I looked alcohol was more like the sixth-largest factor. p.12 line 25: You mention Knott et al as a study suggesting a protective effect of alcohol for type 2 diabetes. In fact, this study casts doubt on the relationship and their data fit your argument better. When they account for some selection biases, they find no evidence of protection in men though they do in women. However, they note the paucity of strong studies controlling for abstainer bias and suggest if there were more it might well be there would be no significant protection observed for either gender. You later in the discussion suggest that you have found evidence that drinking above low-risk drinking guidelines is associated with reduced insulin resistance. But I don't think that is quite right - not all of your subjects would have been drinking above low-risk drinking

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	guidelines I think unless I'm missing something? If they were selected as all drinking in excess of low-risk drinking guidelines you should make that very clear (if so your UK guidelines are unbelievably low). P12 line 52: Your discussion of the strength of the association between drinking and cancer is much too weak. You might look at the recent statements by the US College of Oncologists in the Lancet about the causal association. WHO's International Agency for Research on Cancer has classified alcohol as a class I carcinogen along with asbestos and tobacco for several decades. Causal mechanisms involving the action of acetaldehyde, alcohol's first metabolite, have been identified for cancers of the digestive system. So 'thought to be associated with cancer" is too weak and mechanism 'unknown' inaccurate. P15 line 14: a 'durable effect' claimed then contradicted at line 30! I think your second statement is correct i.e. you have shown evidence of a temporary benefit. p15 Line 32: data do not 'represent and important public health message" – perhaps better English to say they suggest or the results provide the basis for etc.

REVIEWER	Marc Saez Research Group on Statistics, Econometrics and Health (GRECS) University of Girona, Spain CIBER of Epidemiology and Public Health (CIBERESP), Spain
REVIEW RETURNED	14-Jan-2018

GENERAL COMMENTS	The authors try to assess the effects of short-term abstinence on biochemical and physiological parameters, as well as on longer term drinking behaviour. However, they have only had partial success in achieving their objectives. In fact, I have some comments, all of them major. Major comments 1.- Although the authors try to justify it in the Discussion section, it is not clear to me why they did not randomize the individuals. For example, before the indicated ethical problems could have used a randomized clinical trial with successive treatment design or other alternative designs. 2.- In any case, and more in the absence of randomization, I do not understand why the authors have not adjusted the results in a multivariate model, controlling, at least, the observed confounders and / or the modification of the effect (interactions) The authors should repeat the analyzes using multivariate models to adjust the covariates.

VERSION 1 – AUTHOR RESPONSE

We thank the reviewers for taking the time to read our manuscript and their comments aimed at improving the quality of the paper.

Editorial Requests:

- Please revise your title to indicate the research question, study design, and setting. This is the preferred format of the journal.

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The title has been changed to “Short-Term Abstinence from Alcohol and Changes in Cardiovascular Risk Factors, Liver Function Tests and Cancer-Related Growth Factors: A Prospective Observational Study”.

- Does your study design permit the use of 'cause and effect' language in your paper? You say you are looking at the "effect" of alcohol consumption in the title and elsewhere but you appear to be using an observational study design that only allows you to look at associations, not effects.

This use of “effect” has been modified in the title and throughout the manuscript, and replaced with phrases describing an “association”.

- The introduction section could be improved. It should include more references (for example, the third paragraph). Please expand on the background literature on this topic and the rationale for carrying out the study.

The introduction has been expanded, and further references added.

- Can you please elaborate on you how you calculated that a sample size of 47 for the control group was needed? You should provide enough information so that other researchers/ readers could easily reproduce your sample size calculation.

The control group was recruited after data from the abstinence group was analysed. These data were used to calculate the required sample size for the control group. This calculation determined that the following sample sizes were required to detect statistically significant differences of the same magnitude (80% power, alpha 5%, 2-sided test): HOMA score n=47, weight n=21, VEGF n=31, EGF n=30. This detail has been added to the manuscript (page 6, paragraph 2).

- Can your paper please include measures of clinical significance? (e.g. by adding effect sizes to the results section).

Effect sizes have been calculated, and added to Table 1.

- Please add a statement to the methods section confirming that you obtained written informed consent from participants.

This statement has been added (page 5, paragraph 3).

Reviewer: 1

1. This is a confirmation prospective (one month blood and other measurements and 6-8 months telephone questionnaire).
2. The number of authors is too great (17) for the number of participants included (97 drinkers and 48 controls, those that continued alcohol consumption). Thus there was no randomization and patients were divided in groups according to their statement that they will or will not keep on drinking). Personally, I do not know if it is very ethical not to do everything to persuade the participants to abstain from drinking and use them as a control group.
3. The authors report that the participants come from a single center (probably the Institute for Liver and Digestive Health, Division of Medicine, Royal Free Campus, Rowland Hill Street, London NW3 2PF, UK), but the authors come from 9 clinics, including an anesthesiology clinic from USA (12. McGovern Medical School, University of Texas Health Science Center at Houston, Texas 77030, USA). There is disequilibrium here.

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All of the authors merit authorship of this manuscript based on ICMJE guidance. That is, that these individuals each made a substantial contribution to conception, design, acquisition of data, analysis or interpretation of the study. Additionally, each individual participated in review and final approval of the manuscript. Although we acknowledge the number of authors is large, many of the researchers were not in full or part time research but agreed to help with the study intermittently.

The specific contribution of each author is stated in the Acknowledgements (page 19): "GM contributed to study design, participated in data collection, wrote the analytical plan, and drafted and revised the paper. He is guarantor. SM participated in study design, participated in data collection, and drafted and revised the paper. AC and TKB analysed the data, and drafted and revised the paper. CS participated in study design, and revision of the paper. MR, SAK, AJ, CC, JM, AG and TH participated in data collection and revision of the paper. CJ, RS, DN and RJ contributed to study design, and revision of the paper. KM supervised the study, contributed to study design, participated in data collection and drafted and revised the paper."

4. “Participants were assessed at baseline and after one month”, thus the presented results represent changes that happened in one month of abstinence. The authors should include in the title the words “short time”.

We agree, and this has been amended in the title.

5. Are there any practical implications of the study results?

These findings have public health implications on alcohol guidance, and support the recent revision in downwards of alcohol weekly units in UK, Australia and Canada.

Reviewer: 2

I like this paper a lot. I think it contributes well to an emerging literature about the potential benefits of temporary abstinence from alcohol. I have a small number of general observations and a few specific suggestions on small issues that can be tidied up and I think improve the final product a little.
General comments:

1. As you acknowledge, there was no randomisation. However, it is hard to think of a way in which intention to abstain or not abstain in itself could inadvertently lead to significant changes in the various biomarkers used. I think this is a good, pragmatic and convincing design to test your hypotheses.

The groups were actually recruited sequentially, and this has been clarified in the text. The first group recruited was the abstinence group, and based on these data a power calculation was performed to calculate the size of the control group. The control group were recruited from the same population, using the same method of email invitation.

2. I would have liked more information about the sample and exactly how they were recruited. You state that it was by email. It seems unusual to be able to locate so many people planning to give up alcohol for a month. How was the invitation to participate worded? Did you ask people to volunteer who were planning to abstain or, alternatively, to continue drinking? Was recruitment related to a Dry January or similar campaign?
Were participants paid for their time?

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We have access to email lists across University College London and Queen Mary University of London, and were permitted to send email invitations to over 2,000 individuals advertising the study. The study was advertised as a 'health check' as well as an assessment of drinking habits. Subsequent recruitment was by telephone follow-up. The recruitment of the abstinence group was related to 'Dry January', but the control group was recruited subsequently. Participants were not paid for their time, but were provided with refreshments, since the blood tests were performed fasted.

3. I think your findings re insulin resistance and drinking are particularly interesting.
4. Another strength of the study you might stress is the very high follow-up rate. This seems highly unusual - almost unbelievable! I assume you had a captive population or paid them a lot? I would have liked more details to get a sense of how that was achieved.

The high follow-up rate may represent the fact that the majority of participants were University or Hospital staff, with possibly higher than average educational attainment, and therefore highly engaged with the study.

Specific points
- P4 line 7: You say alcohol is the 3rd largest risk factor in the GBD estimates but you cite a 2009 paper. The rank of alcohol has jumped around a lot in recent years with more recent estimates and their updates. Please check for the latest - last time I looked alcohol was more like the sixth-largest factor.

This has been updated in the manuscript (page 4, paragraph 1): "Globally, alcohol is the seventh leading risk factor overall in terms of disability-adjusted life years (DALYs), and is the leading risk factor globally in working age individuals (ages 15-59). Moreover, alcohol use attributable DALYs have increased by over 25% in the last 25 years."

- p.12 line 25: You mention Knott et al as a study suggesting a protective effect of alcohol for type 2 diabetes. In fact, this study casts doubt on the relationship and their data fit your argument better. When they account for some selection biases, they find no evidence of protection in men though they do in women. However, they note the paucity of strong studies controlling for abstainer bias and suggest if there were more it might well be there would be no significant protection observed for either gender.

We apologise for this oversight. The correct study to cite is Baliunas et al, which has been added in addition to the Knott paper.

- You later in the discussion suggest that you have found evidence that drinking above low-risk drinking guidelines is associated with reduced insulin resistance. But I don't think that is quite right - not all of your subjects would have been drinking above low-risk drinking guidelines I think unless I'm missing something? If they were selected as all drinking in excess of low-risk drinking guidelines you should make that very clear (if so your UK guidelines are unbelievably low).

This is correct, the vast majority, but not all, were drinking above low risk guidelines (it was not an a priori inclusion criteria). The text has been modified in the discussion reflect this (page 15, paragraph 1)– the sentence "Our data suggest that alcohol use above recommended guidance markedly increases the risk of the risk of type 2 diabetes" has been replaced with "Our data support a positive association of moderate-heavy alcohol use with an increased risk of type 2 diabetes".

- P12 line 52: Your discussion of the strength of the association between drinking and cancer is much too weak. You might look at the recent statements by the US College of Oncologists in the Lancet about the causal association. WHO's International Agency for Research on Cancer has classified

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alcohol as a class I carcinogen along with asbestos and tobacco for several decades. Causal mechanisms involving the action of acetaldehyde, alcohol's first metabolite, have been identified for cancers of the digestive system. So 'thought to be associated with cancer" is too weak and mechanism 'unknown' inaccurate.

The text has been modified in the manuscript to reflect this (page 15, paragraph 2). "Alcohol is causally related to the development of several cancers, including the digestive tract, nasopharynx, liver and breast, and is classified as a class I carcinogen.[4,16] The increased risk caused by alcohol persists even at low-levels of consumption. The mechanism of mutagenesis is thought to relate to direct effects of the alcohol metabolite, acetaldehyde[4]."

P15 line 14: a 'durable effect' claimed then contradicted at line 30! I think your second statement is correct i.e. you have shown evidence of a temporary benefit.

The initial comment of a 'durable' effect was in reference to the behavioural data at 6-8 months. This was to draw a distinction with the remainder of the data which is short-term (1 month). We have softened this in the text – the phrase 'durable effect' has been deleted (page 18, paragraph 1): "This study demonstrates a change in drinking behavior at 6-8 months following a short-term period of abstinence, albeit we cannot exclude the behavioural effect of participation in the study."

- p15 Line 32: data do not 'represent and important public health message" – perhaps better English to say they suggest or the results provide the basis for etc.

This phrase has been deleted.

Reviewer: 3

The authors try to assess the effects of short-term abstinence on biochemical and physiological parameters, as well as on longer term drinking behaviour. However, they have only had partial success in achieving their objectives. In fact, I have some comments, all of them major.

Major comments

1. Although the authors try to justify it in the Discussion section, it is not clear to me why they did not randomize the individuals.
For example, before the indicated ethical problems could have used a randomized clinical trial with successive treatment design or other alternative designs.

As stated above, this was an observational study involving two groups of subjects, one group who stopped drinking alcohol and one group that did not. We do not believe that the intention to stop drinking or continue drinking for the purposes of this study would be a significant confounder, and for ethical reasons we did not randomize to abstinence or continued drinking.

2.- In any case, and more in the absence of randomization, I do not understand why the authors have not adjusted the results in a multivariate model, controlling, at least, the observed confounders and / or the modification of the effect (interactions). The authors should repeat the analyzes using multivariate models to adjust the covariates.

Initially, as we state in the manuscript, we undertook a non-parametric approach to account for lifestyle variables, since many were distributed with a negative skew. However, we have now also added a multivariate model to account for the effect of lifestyle factors. The Simple Lifestyle Indicator

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Questionnaire (SLIQ) provides a raw and a categorical score for exercise and diet. Since the distribution of raw scores was skewed (as mentioned above), the relationship between the raw scores and log odds of the dependent variable were not linear, and the assumptions for the multivariate model were not met. Therefore, changes in categorical score (better/same/worse) were used for the model. Additionally, the cumulative change in exercise and diet score was used, as changes in individual exercise and diet components did not provide enough changes in category for robust assessment.

The multivariate analysis is shown in Table 2 (page 13). This multivariate analysis demonstrated that abstinence remains a significant predictor of 'clinically significant' improvement in primary and secondary biological endpoints, independent of changes in diet and exercise.

VERSION 2 – REVIEW

REVIEWER	Prof. Dr. Marc Saez Research Group on Statistics, Econometrics and Health (GRECS), University of Girona, Spain and CIBER of Epidemiology and Public Health (CIBERESP), Spain
REVIEW RETURNED	11-Mar-2018

GENERAL COMMENTS	The authors have answered quite well not only my comments, but also those of the other reviewers. They have also incorporated a large part of them in the new version of the manuscript.. I have no further comments.

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