Author's response to reviews

Title: Influence of smoking and diet on glycated haemoglobin and 'pre-diabetes' categorisation: a cross-sectional analysis.

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Author's response to reviews: see over
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Influence of smoking and diet on glycated haemoglobin and ‘pre-diabetes’ categorisation: a cross-sectional analysis.

Thank you for giving us the opportunity to make further improvements to this manuscript. We are grateful for the reviewers comments, which have substantially increased the quality of our manuscript:

We have answered all the reviewers’ comments (below) and edited the manuscript in several places, which are highlighted in yellow, and have also attached a detailed set of responses.

With these edits in place we hope that this improved manuscript is now ready for publication.

Prof Mike Lean, on behalf of the authors

Responses to reviewers
The authors would like to thank the reviewers for their helpful and constructive comments on our work.

Reviewer #1
Discretionary Revisions
L126 consider replacing ‘variables’ with ‘participants’
From the variety of dietary variables available on the Scottish Health Survey dataset, only a subgroup relevant to our hypothesis was selected. The authors are describing this variable selection process rather than the participant selection process at that point.

L181 Consider changing ‘approaching 3’ to ‘more than 2 fold’ for 2.63 OR
Amended in the revised manuscript

L249 consider re-wording ‘secure’
The line now reads L250: smoking on protein glycation and diabetes progression is not so clear.

L251 consider rephrasing ‘7% higher’ for HbA1c (%)
The line now reads L252: a relative 7% increase in HbA1c compared to non-smokers (as originally reported by the authors of the relevant reference)

L316 consider replacing ‘only’ with ‘only, or even the main’
Amended in the revised manuscript

Minor Essential Revisions
L39 please add a p value to all comparisons made in the abstract
Amended in the revised manuscript

L39 ‘Smokers twice as likely’ is this data after correction for age, BMI etc.
The line now reads L39: Smokers were twice as likely to have HbA1c in the ‘pre-diabetic’ range (5.7-6.4%) (adjusted model)

Blood glucose fluctuations are assumed to be minimal (L82) in non-diabetic participants. However Wainer (2008, Chance, vol 21 no.4 56-71) illustrate the importance of variability in diabetes.
The authors agree with the reviewer that glucose fluctuations are substantial in diabetes. Increased variability in blood glucose concentration is also important in pre-diabetes, contributing to diagnostically important elevations of HbA1c above thresholds. However, individuals with HbA1c<5.7% (as proposed in the manuscript) are less likely to experience large blood glucose fluctuations and related metabolic abnormalities. Thus, in this category, lesser (non-glycaemic) influences on HbA1c may be relatively more important.

L162-164 There are 10 adjuster described here. Please comment on the possibility that small errors in some adjusters explain the significant differences observed.

All associations were tested, firstly, in an unadjusted model and if significant they were then inserted in the fully adjusted model. That ensures that all significant associations are
indeed true and the likelihood of over-adjustment explaining significant differences is minimal.

L170-171 The data in Table 1 appears to be no different between the groups (5.3±0.4 non-smokers vs 5.4±0.4 smokers (%HbA1c). Is there a way to present this data that illustrates the difference?

Figure 2 has now been replaced with a figure depicting HbA1c levels according to smoking status. The illustration of the difference is clearer in the figure than in the table.

L287-290 The effect of vegetables is extrapolated here to mean antioxidants. This generalisation is not trivial and would need to be substantiated.

Fruit and vegetable intakes were used in this study as proxy-measures for antioxidant intake, as they represent the main dietary sources of antioxidants. It is indeed possible that changes in fruit and vegetable intake may well affect fibre, mineral and other micronutrient intakes. A relevant section has been added in the limitations section.

L332-333 and an increased consumption of fibre, minerals and other micronutrients.

To comply with data deposition standards of publication, please add a link to the data deposition and the experimental design deposition (e.g. www.clinicaltrials.gov)

Website link was added in L106

Major Compulsory Revisions

1. Clarify if the hypothesis is that smoking causes diabetes or biases the HbA1c measurements.

Previous longitudinal studies have documented increased risk of diabetes in smokers and passive smokers, but the mechanism is unclear.

The primary hypothesis of the study is that smoking (a proxy of oxidative stress) is associated with increased protein glycation, as measured by HbA1c, in normoglycaemic individuals. The cross-sectional nature of our data precludes direct claims for causality, but laboratory evidence confirms that oxidative stress does increase protein glycation.
This mechanism will move some individuals from normal to pre-diabetic and diabetic ranges of HbA1c, or accelerate progression to diabetes. We have clarified the hypothesis along these lines in the manuscript, lines 89-90, 96-98.

2. L44-46 The conclusion that ‘this study adds evidence to the neglected link between oxidative stress and protein glycation’ is overreaching. No evidence is presented to support the ‘neglected link’. Furthermore, the generalisation to oxidative stress and glycation is not warranted by epidemiological retrospective study.

L45-46 now reads: This study adds evidence to relate smoking (a proxy for oxidative stress) to protein glycation in normoglycaemic subjects, with implications for individuals exposed to ROS and for epidemiological interpretation of HbA1c.

3. The assumption that glucose levels do not differ among non-diabetic participants needs to be substantiated. It forms the basis of the conclusion smoking affects HbA1c glycation, but no evidence is presented. In my experience, there is substantial difference both in fasting and postprandial glycaemia within the non-diabetic range. Indeed, the progressive nature of the disease suggests that would be the case (Tabak et al 2012 Lancet 397 2279-90)

We did not intend to suggest that glucose levels do not differ among non-diabetic participants (and did not say, or assume, that). It is important only to reflect that the normal range of fasting glucose is remarkably narrow, usually 3.6-5.6 mmol/l, rising only to about 7-8 mmol/l post-prandially, while among people with diabetes experience much wider ranges and greater fluctuations.

We have commented above to Reviewer 1 on blood glucose fluctuations, related to line 82.

Reviewer #2

Minor Essential Revisions
L78 and in a separate study (comprising of x subjects)-this sentence does not read particularly well so could be revised
The line now reads L77-78: HbA1c relates strongly to tissue damage in T2DM patients and it has also been found to predict ....

L93 consider revising the word ‘unimportant’ here
This has now been changed to ‘are unlikely to have a major impact’

L281 blood glucose concentration- preferred term rather than levels?
Amended in the revised manuscript

L317: our data does?
‘Data’ being plural (of the word datum), we have retained the correct English: ‘our data do’.

L338 and inversely correlated with vegetable intake? Perhaps this makes the sentence more complete
Amended in the revised manuscript

We hope that our responses satisfactorily resolve the issues raised by the reviewers, and that our improved paper is now ready for publication.

Best wishes
Prof Mike Lean