Author’s response to reviews

Title: Predictive value of serum testosterone for type 2 diabetes risk assessment in men

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Author’s response to reviews:

Responses in italics

Reviewer #1:

1. It is not clear how the present study group was selected from the previously existing cohorts of FAMAS and NWAHS. A schematic representation showing subject selection for the baseline and follow-up study should be included in the methods section
Please see the following changes on page 7:

A flow of the study population at each stage of the MAILES Study, with the numbers of participants drawn from the respective stages of FAMAS and NWAHS has been published [22].

2. What are the inclusion and exclusion criteria for the study? It is not sufficient just to mention that ‘eligible male participants’ were included in the study.

For both points 1 & 2, please see the substantial changes made on pages 5-8, now describing in detail the various inclusion and exclusion criteria.

3. It is also important to mention if the subjects were free of diabetes at baseline and if their diabetic status was confirmed by means of self-reporting or by testing their fasting glucose levels. Also the baseline Fasting plasma glucose values of those who developed and did not develop type 2 diabetes can be provided

T2D at baseline and follow-up was determined using the same algorithm for case definition. All participants with diabetes at baseline were excluded from the study. The following changes were made in the abstract and on page 8 for greater clarity:

‘T2D at baseline and follow-up was defined by self-reported medically…..’

Table 1 now presents baseline FPG by T2D status at follow-up (page 26)

4. Table1. It would be more informative if the mean values of the biochemical parameters like triglycerides, HDL-C, HbA1c, testosterone levels at baseline and after followup are provided between the two groups (those who developed and did not develop type 2 diabetes).
We have added the requested baseline information to Table 1. However, we believe that the additional information requested by the reviewer would result in a very large and unclear Table. Furthermore, risk factor values at follow-up are not relevant because they are not included in any of the risk models we studied (i.e. no predictive value).

5. Another sub-analysis could be performed to see if people in the highest to lowest tertile of Testosterone at baseline respectively had the highest to lowest risk of developing type 2 diabetes after the follow up period.

We explored this hypothesis for the reviewer, and the results are presented below:

Confirms that the lowest tertile of testosterone at baseline have highest chance of developing diabetes (13.2% vs 8.8% vs 5.4%)

Testosterone tertiles * diab2_combined_dichot2_m2 Crosstabulation

<table>
<thead>
<tr>
<th></th>
<th>No diabetes</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone tertiles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowest tertile</td>
<td>433 (86.8%)</td>
<td>66 (13.2%)</td>
</tr>
<tr>
<td>Middle tertile</td>
<td>455 (91.2%)</td>
<td>44 (8.8%)</td>
</tr>
<tr>
<td>Highest tertile</td>
<td>474 (94.6%)</td>
<td>27 (5.4%)</td>
</tr>
</tbody>
</table>
| Total              | 1362        | 137      | 1499

% within Testosterone tertiles: 90.9% 9.1% 100.0%
### Chi-Square Tests

<table>
<thead>
<tr>
<th></th>
<th>Value</th>
<th>df</th>
<th>Asymp. Sig. (2-sided)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pearson Chi-Square</td>
<td>18.585a</td>
<td>2</td>
<td>.000</td>
</tr>
<tr>
<td>Likelihood Ratio</td>
<td>18.815</td>
<td>2</td>
<td>.000</td>
</tr>
<tr>
<td>Linear-by-Linear Association</td>
<td>18.476</td>
<td>1</td>
<td>.000</td>
</tr>
<tr>
<td>N of Valid Cases</td>
<td>1499</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. 0 cells (0.0%) have expected count less than 5. The minimum expected count is 45.61.

However we do not wish to present these results in our study given that they detract our novel finding showing the optimal cut-off for low serum testosterone for predicting incident T2D in men.

6. There is a risk of overestimating the effects in sub-analyses as the sample size is low and this should be mentioned as a limitation of the study.

There were no sub-group analyses per se. The sensitivity analyses (Models 6-8) are only used to describe the stability of existing models rather than to discover or report new relationships. They are descriptive rather than inferential.

7. The proportion of non-responders and dropouts is high in this study. Were the testosterone levels and other biochemical characteristics of these subjects different from the study participants?

The risk of selection bias from non-respondents is low. We have published (cited on pages 6 & 7) papers reporting on the representativeness of both FAMAS and NWAHS.

The risk of withdrawal bias from loss to follow-up is also low, and favours underestimation of predictive effects in the models.
The following changes were made on pages 7 & 8):

Reasons for loss to follow-up (non-respondents) include death (n=99), too ill to participate (n=39), withdrew from the study (n=141), were unable to be tracked due to changes in contact details (n=77), and refused to take part in MAILES Stage 2 due to work-related and personal reasons (n=169). Non-respondents in MAILES Stage 2 were more likely than respondents to report diabetes, cardiovascular disease, or depression, or to either not know or to not disclose their chronic-disease status. With regard to their self-reported general health and smoking status, non-respondents were more likely than respondents to report that they had poorer health and were current smokers or to not to provide information about these factors. Regarding measured health-related risk factors, non-respondents were less likely than respondents to be overweight or obese, or to have central adiposity and a high. Finally, there were no statistically significant differences between respondents and non-responders in means for serum testosterone, fasting plasma glucose (FPG), triglycerides, and high density lipoprotein cholesterol (HDL-C).

8. Some reports argue that men on testosterone replacement therapy men had increased risk of cardiovascular events. This should be discussed in the discussion section

The following changes were made on pages 14 &15):

Conversely, testosterone therapy has been associated with serious adverse events in men. A systematic review of 27 RCTs found that testosterone therapy vs. placebo increased the risk of a cardiovascular-related event in mainly older men (pooled odds ratio was 1.54 [95% CI: 1.09, 2.18]) [56]. However, a more recent systematic review of RCTs in mostly older men found there was an increased cardiovascular risk associated with oral testosterone therapy (pooled relative risk was 2.20 [95% CI: 1.45,3.55]), but not with intramuscular (pooled relative risk was 0.66 [95% CI: 0.28,1.56) or transdermal (gel or patch) testosterone therapy (pooled relative risk was 1.27 [95% CI: 0.62,2.62]) [57]. Further research is needed to establish the safety of specific types of testosterone therapies in specific populations.

Reviewer #2:
• In abstract, kindly provide more results than detailing the methods utilizing the full word limit
We have provided more results, as requested.

• In abstract, please check the sentence (Line number:27) "values (PPV) where…..
This has been corrected to ‘were’.

• It is not clear whether OGTT have been done at baseline to rule out prediabetes?
Please see specific changes to the abstract and methods sections (page 8) clearly stating that T2D at baseline and follow-up was determined using the same algorithm for case definition.

• Dyslipidemia is a risk factor for diabetes whereas treatment with statins can also result in dysglycemia. Considering these diagonally opposite effects, is there any data of those individuals with dyslipidemia?

• How many with dyslipidemia at baseline were treated with statins?

• Was the incidence rate of diabetes different in those treated with statins?

For all three points above:

We accept that these are important research questions worthy of future research. However current use of statins is not relevant in our study because it was not included in any of the risk models we tested (i.e. it has no predictive value).

As described in the abstract:

“We sought to determine whether low serum testosterone, a novel risk factor for T2D in men, adds clinically meaningful information beyond current T2D risk models.”
• In the logistic regression analysis (Model 3), the authors included more covariates, which increases the risk of overfitting the model. Kindly check the potential effects of the number of covariates (for example using propensity score).

Overfitting may be possible. Although we have 100 times more observations than the number of predictor variables, this ratio based rule of thumb is known to have weaknesses and exceptions. We calculated the propensity scores using the 14 predictors in Model 2 (i.e. all predictors except testosterone) and then ran a logistic regression with this propensity score and testosterone as the only two predictors. The saving of 23 degrees of freedom on a sample size of 1499 proved to have little impact – with the p-value for testosterone changing from 0.032 to 0.042.

In the Methods section we have added: “The Nagelkerke R2 was monitored for sudden movement towards 1 which could indicate overfitting.”

In the Results section we have added “with the Nagelkerke R2=0.25”.

In the Discussion we have added the sentence: “While there were only 147 incident cases, the ratio of 100 observations per predictor variable, the relative stability of the AIC and BIC and the fact that the Nagelkerke R2 is much lower than 1 provide no evidence of over-fitting.”

• Whether any data is available to show the effect of statin treatment on testosterone levels?

The risk of confounding or effect modification from an association of statins with serum testosterone level is low. A recent systematic review of RCTs (placebo-controlled) in men, mainly middle aged with hypercholesterolemia, showed that statins lowered testosterone by -0.66 nmol/l (95% CI: -0.14 to -1.18). This small treatment effect has not been shown to be clinically relevant for predicting T2D risk, and is well below the mean difference reported in studies comparing men with vs. without T2D and MetS (please see page 5).
Reference:


• In the discussion please comment on the generalizability of results to other ethnic populations

We have made the following change on page 16:

Further evidence from prospective cohort studies is needed to confirm the generalizability of these findings and the applicability of screening for low serum testosterone in other male populations and specific healthcare settings.

The predictive value of low serum testosterone for T2D risk has shown across a range of male populations and ethnic groups in studies from the United States in both general and Japanese populations, Sweden, Finland, and Australia, as cited on page 5.

We tested the predictive value of serum testosterone in an Australian population for T2D risk models previously validated in populations including Australia, UK, Finland, Germany, USA, and UK.

Editorial Requests:

Ethics:

If your study involves humans, human data or animals, then your article should contain an ethics statement which includes the name of the committee that approved your study.
Included

Consent:
If your article is a prospective study involving human participants then your article should include a statement detailing consent for participation.
Included

Availability of supporting data:
The MAILES cohort data set is not publically available.

Authors Contributions:
Your 'Authors Contributions' section must detail the individual contribution for each individual author listed on your manuscript.
Included