Author's response to reviews

Title: Nine-year incident diabetes is predicted by fatty liver indices: The French D.E.S.I.R. study

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Author's response to reviews: see over
Thank you for the opportunity to submit a revision of our manuscript MS470719884390198: Nine year incident diabetes is predicted by fatty liver indices: the French D.E.S.I.R. study.

We have now complied with the Editorial comments, to include a statement and the name of the ethics committee that approved the protocol. Further, we have followed the format of a recent article in BMC Gastroenterology, and changed the references to comply with the BMC Gastroenterology style.

We have edited the article to improve the style. In fact, the first author is Australian, and a native English speaker.

The comments by the five reviewers are addressed in the following pages. We appreciate their careful reviews, and the fact that you take the trouble to have five reviewers.

We hope that the manuscript is now suitable for publication in BMC Gastroenterology, but we are certainly willing to make changes to the text if the Reviewers have other recommendations to improve our manuscript.

Yours

Beverley Balkau
Reviewer 1

I think the manuscript can be improved by discussing the high OR for T2DM which was observed in women with a FLI >70 and/or by discussing the utility of such indexes as compared with traditional risk factors.

While the ORs for women are higher than those for men, they do not differ significantly. A test has been provided, and the results are now indicated in the text, page 10-11.

"Comparing the highest quartile group of these indices with the group including individuals in the three lower quartile groups, after adjustment for other factors associated with incident diabetes, the two indices provided similar odds ratios - for men of the order of 2, and for women, 4. However, the odds ratios did not differ statistically between men and women, (FLI: P = 0.2 and NAFLD-FLS: P = 0.1)."

The utility of these scores is commented on below, see 2. and in the text of the article. Thank you for this suggestion, as I believe that it improves our manuscript.

The authors present some of these results in the table but there is no discussion.

We did not discuss in the results section, all of the lines in Table 2 - we wanted to provide the reader with the possibility to evaluate the odds ratios after adjustment by different risk factors.

Major comment:
1. The risk for diabetes was particularly high in women with a FLI >70 (OR 37). This gender related effect deserves additional analysis and discussion, i.e. in relation to an effect of the menopause.

As stated above, while the ORs for women are higher than those for men, they do not differ significantly, and further, they do not differ significantly for women under and over 50 years.

We have added the following on page 11

"Comparing the highest quartile group of these indices with the group including individuals in the three lower quartile groups, after adjustment for other factors associated with incident diabetes, the two indices provided similar odds ratios - for men of the order of 2, and for women, 4. However, the odds ratios did not differ statistically between men and women, (FLI: P = 0.2 and NAFLD-FLS: P = 0.1)."
2. Please, make clear which is the plus value of the FLI index or of the NAFLD-FLS respect to common predictors of T2DM such as familiarity for the disease. Do the indexes identify different samples of high-risk individuals as compared with traditional risk factors?

We have already published a score for incident diabetes [reference 16] which by definition is the best possible score to predict diabetes for our data.

The strongest single predictor for diabetes is previous high fasting glucose levels - which are not included in the FLI, but after adjusting for baseline glucose, the FLI remains predictive.

For the NAFLD-FLS, fasting glucose is only included as part of the metabolic syndrome, and again the NAFLD-FLS is predictive of incident diabetes after adjustment for fasting glucose at inclusion.

We have now included in the text the c-statistic, which evaluates the discrimination between those who become diabetic and those who do not, to compare the fatty liver indices and the D.E.S.I.R. diabetes risk score.

In the methods section, page 8-9 we have added:

“The c-statistic and the model goodness-of-fit Hosmer-Lemeshow tests were evaluated for the two indices, and compared with the predictive model for incident diabetes that we have already published [16]. The D.E.S.I.R. diabetes risk score includes, for men: waist circumference, smoking, fasting glucose and GGT, and for women: BMI, diabetes in the family, fasting glucose and triglycerides.”

In the results, page 11:

“The fully adjusted FLI provided a reasonable discrimination of cases and non-cases of incident diabetes, with c-statistics of 0.74 in men and 0.84 in women and for the NAFLD-FLS the c-statistics were 0.74 and 0.83, respectively; the models provided a reasonable fit according to the Hosmer-Lemeshow test, with all tests non-significant.

As expected, the models we have already published, which included the best combination of clinical and biological factors to predict diabetes, had higher c-statistic values, 0.85 in men and 0.92 in women.”
The “plus value” for these fatty liver indices is that they provide a marker for the generalist or the hepatologist, to envisage screening for diabetes - or for pre-diabetes. We state as the last sentence of the article:

"The novel finding of our study is that these two recently published indices of liver fat are predictive of diabetes in both men and women. The association was independent of traditional risk factors for diabetes such as glucose, diabetes in the family, insulin, , sedentarity, smoking and alcohol consumption, suggesting the clinical interest of these indices to better identify patients consulting hepatologists who are at high risk of progression to diabetes."

The diabetes risk score and the fatty liver risk scores do screen different individuals. We have added to P11:

"The fatty liver indices and the D.E.S.I.R. diabetes score screen different high risk individuals: those with a high score from the D.E.S.I.R. risk score have higher glucose and HbA1c levels but have less adiposity, lower liver function tests and lower insulin levels than those at risk according to the FLI and the NAFLD-FLS indexes,"

Minor comments:
1. Bedogni and not Bedogini (page 3)
   Thank you
2. BMI and not (BM)I (page 3)
   Thank you
3. In the introduction there is no need to talk about CRP or NEFA or TNF-alpha as these measures do not enter the FLI or the NAFLD-FLS scores. May be the authors want to highlight the concept that the NAFLD can be considered the hepatic component of the MetS and that component of the syndrome enter both the indexes. We have removed the references to CRP, NEFA and TNF alpha.
Reviewer 2

The authors should pay more attention to some figures in the Table 2 and Table 3, such as the number of men with fatty liver index #70 should be 277 instead of 270, and the number of women with Fatty Liver Index 20-69 where alcohol < 20 g/day should be 421 instead 481 in Table 2.

Thank you for this careful reading of our tables. We have corrected the errors.

The authors should give some explanation if some data were missing in defining NAFLD fatty liver score.

There are no missing data for the FLI, but there are some missing data for the calculation of the NAFLD-FLS, and this has now been noted on page 5 of the manuscript.

"We study the 1861 men and 1950 women who had data available at baseline on glucose, triglycerides, BMI, GGT, waist circumference and known diabetes status at the 9-year examination. For the calculation of the NAFLD-FLS, data is available for 1848 men and 1940 women."

In addition, ‘waist’ in table 1 should be changed into ‘waistline’ or ‘waist circumference’.

Thank you, we have changed this.
Reviewer 3

1) As stated by the authors themselves, definition of fatty liver used in this study was only based on surrogate indexes without any data from scanning device. In the absence of such fundamental direct data on liver state it is hard to give any conclusion on validation of the above indexes of fatty liver to predict incident diabetes. The aim of our study was not to make conclusions about the validity of these indices, but rather to show that they did have the utility of predicting incident diabetes.

2) The utilized NAFLD-FLS index includes many parameters of metabolic syndrome such as central obesity, lipid concentration and plasma insulin concentrations that are per se important predictors for incident diabetes. Therefore, if NAFLD-FLS index could be considered an acceptable index for fatty liver prediction it does not appear to be the same for predicting diabetes. In fact the NAFLD-FLS does include parameters which alone predict diabetes - the metabolic syndrome predicts diabetes, as do its elements. We have studied whether this score also predicts diabetes.

Moreover, although the authors state that “the term in type 2 diabetes does not contribute in our study”, the use of such an index that includes predefinition of presence of diabetes to evaluate incident diabetes is strongly questionable. Because we were studying incident diabetes, no diabetic patients were included at baseline in our analysis. The diabetes term was thus zero for all participants. This is stated on page 7 of our manuscript.

"In our population, as we studied incident diabetes, there were no individuals with diabetes at baseline, so the term in type 2 diabetes does not contribute to the NAFLD-FLS in our study.

3) The new finding of the study is somewhat limited by the knowledge that some previous studies had already demonstrated that aminotransferases correlate to type 2 diabetes and predict incident diabetes per se (The Mexico City Diabetes Study), other than as well as plasma glucose and insulin. The scores used include more variables than just the aminotransferases, as other variables enter the predictive equation. The scores should thus provide a better prediction of fatty liver, and we use this score to predict diabetes.

4) In the present study the authors tried to identify a new predictor of diabetes useful for general population; however they did not study a general population, but only volunteers; this could have introduced some selection-bias. One of the difficulties with all epidemiological studies is that they include volunteers, thus almost all epidemiological studies have this limitation. We do not aim to determine prevalences, but relations between risk factors and later diabetes. We believe that the prospective relations that we determine can be extrapolated to other populations.
5) The population was not screened for fundamental biochemical parameters such as markers of viral hepatitis; this limitation clearly affects the strength of reported results. We do not have information about possible viral hepatitis, but we believe that in a clinical setting the physician does not always have this information, and so these fatty liver indices are still useful.

6) The Authors stated that population was evaluated according to the degree of physical activity and to where the activity was done (see Measures of Inclusion Section page 5); no criteria utilized to define physical activity were, on the contrary, reported; in the absence of definition, the interpretation of data as reported in Table 1 (Intense physical activity) and in Results Section (see page 8) is strongly limited. We have now included more information about these variables on page 5:

"Smoking habits (current smoker or not), alcohol consumption (gram per day of alcohol from wine, beer, cider, spirits) and degree of physical activity (at home, at work and sport, each one coded on a four level scale) were assessed using a self-administered questionnaire. Intense physical activity was coded if an individual had a score of four on any of the three items (at home, at work or sport), or a score of 3 for sporting activity and 3 for physical activity either at home or at work."

The same for the smoking habits: what criteria were utilized? (Smoker non smoker?; other?) Smoking habits have now been defined in the methods as either current smoker or not, see above.

7) The studied population shows clear unbalance as far as Women/Men distribution according to Fatty liver index (see Table 1). Actually, 73% of women showed a fatty liver index < 20 vs 32% as obtained in men. This point should be carefully discussed. There is a difference in the distribution of the FLI between men and women. We have added the following in the text:

"As expected, the distribution of the FLI differed between men and women, with fewer women having a high FLI."

While tables 1 and 2 present data according to the same classes of FLI for men and women, in Table 3, sex specific quartiles are used, so that the two indices can be compared. The same relations are seen with this division of the FLI into sex-specific quartiles as in Table 2.

8) Data on FLI classes are shown as mean +/- SD; this form of presentation of data is questionable in the absence of data on distribution. Whatever the distribution of the variables, the mean and SD of the data is the true mean and SD of the population, even when the data is skewed. Testing has used transformed data for the skewed variables.

The manuscript needs important revision for English. We have carefully revised the manuscript to simplify the English and to make it clearer and easier to read.
Reviewer 4

Major Compulsory Revisions
The authors conclude that the two indexes studied predict the incidence of diabetes. However, I have doubts with respect to the utility of the HAFLD-FLS index. The authors should clarify this aspect since the utility of this index is limited by the inclusion of diabetes and fasting insulin in its definition.

The NAFLD-FLS index is complicated, and it includes hyperglycaemia twice (once in the IDF syndrome and again as a separate item, as diabetes) and it includes AST on its own and as a ratio with ALT. Additionally, insulin is generally not available in routine biology, limiting the utility of this score.

However, despite these limitations, for the purpose of our manuscript, the NAFLD-FLS is predictive of incident diabetes.

I do not understand how the presence of diabetes can be predicted if being diabetic is included in the definition. Because we were studying incident diabetes, no diabetic patients were included at baseline in our analysis. The diabetes term was thus zero for all participants. This is stated in the text, page 7:

"In our population, as we studied incident diabetes, there were no individuals with diabetes at baseline, so the term in type 2 diabetes does not contribute to the NAFLD-FLS in our study."

In the limitations the authors recognize the absence of imaging tests for the follow up of fatty liver. The doubts presented are: Is the determination of these indexes sufficient to suspect the progression of fatty liver? Progression of fatty liver is a question that our study was not intending to answer. Scans would be required to be able to answer this question.

Perhaps it would be interesting to at least do abdominal echographies to confirm the progression of fatty liver at the same time as performing the indexes to thereby support the importance of these indexes. The aim of our study was not to validate the indices, but to show that they did indeed predict incident diabetes.

It is strange that the authors did not consider the use of abdominal echography which is very usual, inexpensive and non invasive. The authors should address these questions. Our data come from routine health check-ups and abdominal echography is not included in these examinations.

Discretionary Revisions
The authors show the incidence of diabetes at the end of the nine years of follow up. Since the patients were examined every three years, I believe that it would enrich the manuscript if the authors were to report the incidence of diabetes at each visit.
We have now included the incidence of diabetes at the intermediate examinations, in Table 1, as well as the cumulated incidence over the 9 years of the study. This is commented on in page 9 of the manuscript: "Incident diabetes increased across the FLI groups, $P < 0.0001$ for both men and women, and despite the small numbers of incident cases, the three-yearly incidences also increased across FLI groups."
Reviewer 5

I. Major Compulsory Revisions

1. The indices used in this study still require validation in populations outside of the populations that they were developed from. In addition, the performance characteristics of the indices, the Fatty Liver Index in particular, leave room for misclassification considering a positive likelihood ratio of only 4.2 for predicting fatty liver.

We agree that these two indices need validation outside of the populations in which they were developed, which are quite specific populations. Further, we entirely agree, there is room for misclassification.

2. The original reports for the two indices suggest cut-off scores for the diagnosis of fatty liver disease - a FLI \( \geq 60 \) and a NAFLD-FLS \( > -0.640 \). It is thus the understanding of this reviewer that the question the study wanted to answer was whether NAFLD as diagnosed using the two indices would be predictive of incident diabetes. An affirmative answer to this research question would be clinically relevant as screening for diabetes might be advocated in patients diagnosed with NAFLD. The analysis that would have been ideal would have been to divide the patients into those with NAFLD and those without NAFLD based on the cut-offs for both the FLI and the NAFLD-FLS.

We believe that the scores are continuous scores, and that the cut-points provided are arbitrary - the risk of NAFLD increases as these scores increase. We thus studied three groups for FLI as well as quartiles of both scores, so that the two indices could be compared.

Unfortunately we do not have the data to be able to compare the fatty liver indices and echographic evaluation of NAFLD.

The two groups would then be compared as to the development of incident diabetes. I would submit that the results of the current analyses using quartiles are not at all surprising as these indices contain known predictors of incident diabetes such as triglycerides, BMI, and waist circumference for FLI and metabolic syndrome for NAFLD-FLS. In fact, results to the contrary would be difficult to explain.

The main conclusion from this work is that those with suspected fatty liver, either calculated from these scores or on the basis of clinical observations, should be screened for diabetes and pre-diabetes. This is included in the concluding paragraph:

"The novel finding of our study is that these two recently published indices of liver fat are predictive of diabetes in both men and women. The association was independent of traditional risk factors for diabetes such as glucose, diabetes in the family, insulin, sedentarity, smoking and alcohol consumption, suggesting the clinical interest of these indices to better identify patients consulting hepatologists who are at high risk of progression to diabetes."

10/12
3. In the calculation of the odds ratios for incident diabetes, it would be expected in the multivariate analysis that potential confounders of the association between the indices and incident diabetes be adjusted for. Table 1 shows in addition to the adjustment factors shown in Table 2, variables such as BMI, waist circumference, cholesterol levels were correlated with the FLI and thus should be included as adjustment factors.

As the main risk factors for diabetes in this cohort are obesity (BMI and/or waist circumference) this factor is already included in both indices, so we have not adjusted for these variables.
II. Minor Essential Revisions
1. Biochemical measurements were conducted in several sites using different equipment. Are the results from these different sites comparable? While there were different laboratories, all laboratories were part of the same quality control protocol.