

PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Prediabetes, Elevated Iron, and All-Cause Mortality: A Cohort Study
AUTHORS	Mainous III, Arch; Tanner, Rebecca; Coates, Thomas; Baker, Richard

VERSION 1 - REVIEW

REVIEWER	Christina Ellervik Associate Professor, Chief Physician, PhD Department of Clinical Medicine Faculty of Health and Medical Sciences University of Copenhagen Copenhagen, Denmark
REVIEW RETURNED	05-Sep-2014

GENERAL COMMENTS	<p>The article presents data from a general population cohort in US with 12 years of follow-up. The study shows effect modification of iron overload on mortality risk according to prediabetes vs. normoglycemia. The study is novel and important.</p> <p>I have a few minor comments to this otherwise well-written paper:</p> <ul style="list-style-type: none">- To prove that there is effect modification of iron overload on risk of total mortality by prediabetes: What are the p-values for interaction between prediabetes and iron overload in the analyses made in table 2 and 3, unadjusted and adjusted- Page 8, line 20: were data missing for iron parameters in general, or just for iron overload?- What was the participation rate?- In the results section: could you focus more on writing the results than just stating table legends.- References: Another recent ferritin-mortality paper just came out in Clin Chemistry on mortality risk- Could you provide log-rank p-values and number at risk for figures 1 and 2- Figure 1, abstract and methods: I'm a little confused about how many N's total you had: 81,000,000 or 30,000 or 8,000?- statistics: what is meant by the sentence "For the analyses of mortality, we used sampling weights (specifically, the total MEC and Home examined weight) to calculate prevalence estimates for the civilian noninstitutionalized US population.": could you explain that. Do you mean adjustment for sampling method?- Table 1: what is a weighted sample size of 81,000,000? How do the 81,000,000 sample relate to rest of the paper and to the 30,000 and 8,000 subjects- Table 2 and 3: could you provide N's for events and total- Figure 2: could the phrase "...elevated iron" be changed to "...elevated ferritin and transferrin saturation" in the legend- Figure 2: somewhat more clearly drawn lines and dots to distinguish the different lines would improve the figure: it's difficult to differentiate the lines. Or maybe a text pointing towards the lines or
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	<p>a text above each line would help.</p> <ul style="list-style-type: none"> - What is the power for the overall study objective? Or what is the minimal detectable HR given 80% power for the overall study objective - In the Discussion: Could the authors comment on the finding that the “normoglycemic-iron overload group” didn’t experience an increased risk of total mortality, when previous findings have shown an increased risk for iron overload alone. Does your finding imply that iron overload in normoglycemic individuals is not a hazard? Or don’t you have enough statistical power to study the risk in this group. The point-estimate is above 1.0, but the 95%CI is not significant. For the analysis of effect modification, sample size is crucial in the strata.
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REVIEWER	Eugene D. Weinberg Indiana University. USA
REVIEW RETURNED	06-Sep-2014

GENERAL COMMENTS	<p>The association of excessive/misplaced iron with the several types of diabetes is well established. Pancreatic beta cells are killed by low low concentrations of iron (Masuda, Y. et al Am J Transl Res 2014;6:64-70). The present study provides evidence that mortality is increased more than two fold in pre-diabetics with elevated iron as compared with pre-diabetcs with normal iron. This study strongly reinforces the urgent need to incorporate iron markers in routine physical exams so as to alert individuals to adopt iron reduction procedures.</p>
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REVIEWER	Ralph G DePalma Veterans Administration ORD USA
REVIEW RETURNED	29-Sep-2014

GENERAL COMMENTS	<p>The authors should comment on the J shaped curve related to %TSAT in the references they cited. Either low or high may be associated with increased mortality</p> <p>The authors should comment on the J shaped curve related to %TSAT in the references they cited. Either low or high may be associated with increased mortality</p> <p>The authors should comment on the J shaped curve related to %TSAT in the references they cited. Either low or high may be associated with increased mortality. Expanding the section on discussion of phlebotomy effects, briefly in diabetes per se would add to clarity.</p>
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REVIEWER	Leo R. Zacharski Geisel School of Medicine at Dartmouth College, Lebanon, NH 03756 and the Research Service, VA Hospital White River Junction, Vermont 05009, USA
REVIEW RETURNED	29-Sep-2014

GENERAL COMMENTS	Evidence cited by the authors indicates that prediabetes is common
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but its incidence is under appreciated and its contribution to mortality is controversial. Other evidence indicates that elevated measures of iron status contribute to development of diabetes and also predict increased mortality in the general population. This study was undertaken to test the hypothesis that mortality in pre-diabetes is influenced by concomitantly increased iron measures.

Data on prediabetes and iron status spanning the years 1988-1994 were obtained from NHANES III. Data on all-cause mortality spanning the years 1988-2006 were obtained from the National Death Index of the National Center for Health Statistics.

The authors studied individuals over 40 years entered into NHANES III that did not have a prior diagnosis of diabetes and had HbA1c levels less than 6.5%. Analysis eliminated individuals that died within 3 years of the start of follow-up.

Percent TS levels >50% and ferritin levels >400 ng/mL were considered "elevated".

Several weaknesses of this study are discussed.

The authors found that the existence of prediabetes itself had a small effect on mortality compared to normoglycemic individuals. However, the existence of prediabetes plus an elevated percent transferrin saturation (TS) conferred increased mortality. Mortality was increased even further in individuals having both an increased TS and a ferritin of greater than 400. The conclusion was that elevated markers of iron status signify increased body iron levels that contribute substantially to mortality in prediabetes. This is an important study that should point the way to innovative public health interventions having a high probability of success.

Critique:

1. In the "limitations" section on page 3 they say "We were only able to observe individuals for 12 years." But on page 5, second and third lines from the bottom, they say, "All living survey participants had been observed for at least 146 months." The ambiguity is whether this observation period is the median or the minimum.

2. The "n's" in this study are ambiguous. It is stated that 30,818 persons were examined of which 1,123 were excluded for lack of information on prediabetes, 1,288 lacked data on %TS and 1,288 lacked data on ferritin levels. These were apparently the same 1,288 individuals (page 7, line 20). Mortality data were missing on 15 individuals. Individuals were also excluded who died within 3 years from the beginning of follow-up. The number is not given. Is this the same as the 30.7% indicated as "assumed deceased" in the last line of Table 1? Also in table 1 the number with prediabetes is said to be 23%. Is this 23% of 8,041? In the second line of Table 1 we see the number 81,152,997 but the total number examined was said to be 30,818. It was not clear what the larger number represents.

The number of subjects analyzed in the unadjusted and adjusted models was therefore uncertain.

3. The 50% TS threshold for "elevation" in the sense of being at-risk is well supported. However, ferritins lower than 400 are also at-risk (www.healthiron.com). It is OK to use 400 ng/mL as a threshold for the purposes of this study but this should be indicated in the text as an arbitrary cutoff – or state the reason 400 was chosen.

	<p>4. Table 1 contains the “%” sign in the header and also following certain but not all of the entries below. Perhaps this should be one or the other but not both.</p> <p>5. This reviewer would find it helpful to include p-values as expressions of the strength of comparisons. An editorial issue is whether the word “data” takes the pleural verb form (e.g., “...data were...”)</p>
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VERSION 1 – AUTHOR RESPONSE

Reviewer 1

COMMENT

To prove that there is effect modification of iron overload on risk of total mortality by prediabetes: What are the p-values for interaction between prediabetes and iron overload in the analyses made in table 2 and 3, unadjusted and adjusted

RESPONSE

We have modified the analyses somewhat based on the reviewers comments. We did not compute a one parameter interaction term in the analyses because we felt that it was more useful to examine the relationship between the two independent variables with mortality in the four category analyses. Each of the categories provides a parameter estimate (Hazards Ratio) and a 95% confidence interval.

COMMENT

Page 8, line 20: were data missing for iron parameters in general, or just for iron overload?

RESPONSE

We have modified the manuscript to indicate in the Methods section that within this large, nationally representative, omnibus survey data may be missing on a variety of variables. However, the adjusted Cox regressions use listwise deletion of missing values thereby everyone included in the final regression models has complete data on all variables.

COMMENT

What was the participation rate?

RESPONSE

We have included the information provided by the NHANES on the participation rate.

COMMENT

In the results section: could you focus more on writing the results than just stating table legends.

RESPONSE

We have tried to provide more text regarding the results in the results section.

COMMENT

References: Another recent ferritin-mortality paper just came out in Clin Chemistry on mortality risk

RESPONSE

We have added this reference to the manuscript in the introduction and the list of citations.

COMMENT

Could you provide log-rank p-values and number at risk for figures 1 and 2

RESPONSE

We have made this suggested change.

COMMENT

Figure 1, abstract and methods: I'm a little confused about how many N's total you had: 81,000,000 or 30,000 or 8,000?

statistics: what is meant by the sentence "For the analyses of mortality, we used sampling weights (specifically, the total MEC and Home examined weight) to calculate prevalence estimates for the civilian noninstitutionalized US population.": could you explain that. Do you mean adjustment for sampling method?

Table 1: what is a weighted sample size of 81,000,000? How do the 81,000,000 sample relate to rest of the paper and to the 30,000 and 8,000 subjects

RESPONSE

This comment about the sample size used for the analysis is similar to one made by Reviewer 4. We have added more language to the Methods to try and clarify that the NHANES is somewhat different from many other commonly analyzed cohorts (e.g., Nurses Health Study, Womens Health Initiative) because it is a nationally representative study that uses a complex survey design to provide population estimates of the United States. To be used correctly to make population estimates, like we did in this study, the data needed to be weighted and the complex sampling design needed to be accounted for in the analysis. Consequently, all of the estimates represent the population. Using unweighted numbers to compute proportions will not add to the same numbers as the weighted numbers which have been adjusted for the sampling design. We included the unweighted numbers for context in Table 1 but the analysis is actually based on the population estimates and it is important that the reader focus on the population estimates since the ability to make population estimates is the primary strength of the NHANES and sets it apart from other non-population based cohorts.

COMMENT

Table 2 and 3: could you provide N's for events and total

RESPONSE

We have added the number of events to the results.

COMMENT

Figure 2: could the phrase "...elevated iron" be changed to "...elevated ferritin and transferrin

saturation” in the legend

RESPONSE

We have made this change.

COMMENT

Figure 2: somewhat more clearly drawn lines and dots to distinguish the different lines would improve the figure: it's difficult to differentiate the lines. Or maybe a text pointing towards the lines or a text above each line would help.

RESPONSE

We have modified the figure so that the lines are in color which should help with the contrast between the two groups.

COMMENT

What is the power for the overall study objective? Or what is the minimal detectable HR given 80% power for the overall study objective

RESPONSE

As can be seen when evaluating the number of individuals who died and survived for each of the groups, each cell has at least 125,000 subjects with many cells in the millions. This provides more than 80% power.

Total Alive Dead

Prediabetes 18,668,699 11,431,597 7,237,102
Normoglycemia 61,985,089 47,458,061 14,527,028
Total 80,653,788 58,889,659 21,764,130

Normoglycemia and Normal TS 46,373,562 35,649,283 10,724,279
Prediabetes and Normal TS 13,709,893 8,572,762 5,137,131
Normoglycemia and Elevated TS 1,739,490 1,327,253 412,237
Prediabetes and Elevated TS 283,424 156,790 126,634
Total 80,653,788 58,889,659 21764129.62 |

Normoglycemia and Normal Ferritin 45,100,204 34,132,718 10,967,486
Prediabetes and Normal Ferritin 14,080,167 8,614,683 5,465,483
Normoglycemia and Elevated Ferritin 7,996,011 5,662,576 2,333,436
Prediabetes and Elevated Ferritin 2,969,212 1,818,565 1,150,647
Total 70,145,594 50,228,542 19,917,052

COMMENT

In the Discussion: Could the authors comment on the finding that the “normoglycemic+iron overload group” didn't experience an increased risk of total mortality, when previous findings have shown an increased risk for iron overload alone. Does your finding imply that iron overload in normoglycemic individuals is not a hazard? Or don't you have enough statistical power to study the risk in this group. The point-estimate is above 1.0, but the 95%CI is not significant. For the analysis of effect

modification, sample size is crucial in the strata.

RESPONSE

We have added an extra paragraph to the discussion in regards to this finding. We have also added more information to the Methods indicating that we followed the National Center for Health Statistics guidelines for assessing reliability of estimates. All estimates met the reliability criteria of having the standard error of the population parameter estimate being less than 30% of the population estimate. We discovered in the new analyses that the combined elevated TS/ferritin and prediabetes group exceeded the 30% standard error threshold thereby making those estimates unreliable. Consequently, we have removed those analyses from the manuscript.

3) Reviewer 2

COMMENT

The association of excessive/misplaced iron with the several types of diabetes is well established. Pancreatic beta cells are killed by low concentrations of iron (Masuda, Y. et al Am J Transl Res 2014;6:64-70). The present study provides evidence that mortality is increased more than two fold in pre-diabetics with elevated iron as compared with pre-diabetics with normal iron. This study strongly reinforces the urgent need to incorporate iron markers in routine physical exams so as to alert individuals to adopt iron reduction procedures.

RESPONSE

We have added this citation to the introduction of the manuscript.

4) Reviewer 3

COMMENT

The authors should comment on the J shaped curve related to %TSAT in the references they cited. Either low or high may be associated with increased mortality.

RESPONSE

The reviewer made a very good point about low transferrin saturation. There is a possibility of misclassification bias by considering everyone below 50% of transferrin saturation as normal. Low values may carry mortality risks as well. We modified the methods of the project so that individuals with low values of transferrin saturation, HbA1c and ferritin were excluded. In this way, we were able to more clearly consider elevated values versus normal levels. All of the analyses reflect this new definition of the examined populations and the cut-points for normal were drawn from the literature with appropriate citations added to the manuscript.

COMMENT

Expanding the section on discussion of phlebotomy effects, briefly in diabetes per se would add to clarity.

RESPONSE

We have added more verbiage to the discussion on this point.

5) Reviewer 4

COMMENT

In the "limitations" section on page 3 they say "We were only able to observe individuals for 12 years." But on page 5, second and third lines from the bottom, they say, "All living survey participants had been observed for at least 146 months." The ambiguity is whether this observation period is the median or the minimum.

RESPONSE

We have added language to the Methods and the Discussion to clarify this and make the statements consistent.

COMMENT

The "n's" in this study are ambiguous. It is stated that 30,818 persons were examined of which 1,123 were excluded for lack of information on prediabetes, 1,288 lacked data on %TS and 1,288 lacked data on ferritin levels. These were apparently the same 1,288 individuals (page 7, line 20). Mortality data were missing on 15 individuals. Individuals were also excluded who died within 3 years from the beginning of follow-up. The number is not given. Is this the same as the 30.7% indicated as "assumed deceased" in the last line of Table 1? Also in table 1 the number with prediabetes is said to be 23%. Is this 23% of 8,041? In the second line of Table 1 we see the number 81,152,997 but the total number examined was said to be 30,818. It was not clear what the larger number represents.

The number of subjects analyzed in the unadjusted and adjusted models was therefore uncertain.

RESPONSE

As in our response to Reviewer 1, we have added more language to the Methods to try and clarify that the NHANES is somewhat different from many other commonly analyzed cohorts (e.g., Nurses Health Study, Womens Health Initiative) because it is a nationally representative study that uses a complex survey design to provide population estimates of the United States. To be used correctly to make population estimates, like we did in this study, the data needed to be weighted and the complex sampling design needed to be accounted for in the analysis. Consequently, all of the estimates represent the population. Using unweighted numbers to compute proportions will not add to the same numbers as the weighted numbers which have been adjusted for the sampling design. We included the unweighted numbers for context in Table 1 but the analysis is actually based on the population estimates and it is important that the reader focus on the population estimates since the ability to make population estimates is the primary strength of the NHANES and sets it apart from other non-population based cohorts.

COMMENT

The 50% TS threshold for "elevation" in the sense of being at-risk is well supported. However, ferritins lower than 400 are also at-risk (www.healthiron.com). It is OK to use 400 ng/mL as a threshold for

the purposes of this study but this should be indicated in the text as an arbitrary cutoff – or state the reason 400 was chosen.

RESPONSE

The reviewer's comment is well-taken. We have modified our definition of elevated ferritin to be consistent with that of the study by Adams et al (N Engl J Med 2005;352:1769-1778). This level is a threshold of 300 ng/mL for men and 200 ng/mL for women. This level is also consistent with the thresholds proposed by the Iron Disorders Institute (<http://www.irondisorders.org/>).

COMMENT

Table 1 contains the “%” sign in the header and also following certain but not all of the entries below. Perhaps this should be one or the other but not both.

RESPONSE

We have modified the table in line with this suggestion.

COMMENT

This reviewer would find it helpful to include p-values as expressions of the strength of comparisons. An editorial issue is whether the word “data” takes the plural verb form (e.g., “...data were...”)

RESPONSE

We have attempted to provide this information.

VERSION 2 – REVIEW

REVIEWER	Christina Ellervik University of Copenhagen, Denmark
REVIEW RETURNED	10-Oct-2014

GENERAL COMMENTS	<p>Minor comments:</p> <ol style="list-style-type: none">1. Page 9, line 20: “..at least 100...” do you mean “less than 100”?2. Still, I have difficulties understanding that a sample of 8000 individuals above 40 years with eligible hba1c can rise to 80,000,000 in the analyses. There are some calculations in the methods that needs to be stated more clearly for the reader, and what statistical and epidemiological thoughts, considerations or theory that goes behind this. Have you inferred or imputed hba1c and death on the larger sample? How can you extrapolate from 8000 to a larger sample in a cox regression analysis? And also since “only” 30,000 were examined in the whole cohort, why isn’t the results based on them? Also 80,000,000 is not the whole US-population, but is it the whole US population above 40 years old? Or the US-population above 40 years old in the areas that had a health examination?<ol style="list-style-type: none">a. I found this online for NHANES “A sample weight is assigned to each sample person. It is a measure of the number of people in the population represented by that sample person” (http://www.cdc.gov/nchs/tutorials/nhanes/surveydesign/Weighting/intro_i.htm)<ol style="list-style-type: none">i. Thus, in order for the reader to understand the statistics and the numbers in the article, a more elaborate description in the methods and the statistics is needed, also with references (pubmed or online descriptions).3. In the author comments, the authors write that “number at risk” is provided in fig.1. and fig.2., but I don’t see that4. The percentage of events has been added to the Results section, but not the total number of events in table 2 and 3 (see also next comment, which relate to how many actually go into the analyses 8,000 or 30,000 or 80,000,000): could the total number of events be added (maybe bullet 3 and 4 can be explained by the answer to bullet 2)
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REVIEWER	Leo R. Zacharski Geisel School of Medicine at Dartmouth College and the Research Service, VA Hospital, White River Junction, Vermont, 05009. USA
REVIEW RETURNED	13-Oct-2014

- The reviewer completed the checklist but made no further comments

VERSION 3 - REVIEW

REVIEWER	Christina Ellervik Department of Clinical Medicine Faculty of Health and Medical Sciences University of Copenhagen Copenhagen, Denmark
REVIEW RETURNED	21-Oct-2014
GENERAL COMMENTS	Thanks for the clarification. I have no further comments.