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## Prediabetes, Elevated Iron, and All-Cause Mortality: A Cohort Study

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3 **Prediabetes, Elevated Iron, and All-Cause Mortality: A Cohort Study**  
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5 Running Title: Prediabetes, Elevated Iron, and All-Cause Mortality  
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## ABSTRACT

**Objectives:** Data have indicated low to nonexistent increased mortality risk for individuals with prediabetes, but it is unclear if the risk is increased when the patient has elevated iron markers. Our purpose was to examine the mortality risk among adults with prediabetes in the context of co-existing elevated transferrin saturation (TS).

**Setting:** Data collected by the third National Health and Nutrition Examination Survey 1988-1994 (NHANES III) in the United States and by the National Center for Health Statistics for the National Death Index from 1988-2006.

**Participants:** Individuals age 0 and older who participated in the NHANES and provided a blood sample.

**Primary Outcome Variable:** Mortality was measured as all-cause mortality.

**Results:** Adjusted analyses show that prediabetes has a small increased mortality risk (HR =1.04; 95% CI, 1.00-1.09). Among persons with prediabetes who had elevated TS, they had an increased mortality risk (HR=1.56; 95% CI, 1.12-2.17) compared with those with normal TS levels and normal glucose. Persons who had prediabetes and elevated iron on two markers (TS/ferritin) had an even higher increased hazard ratio for death (2.28; 95% CI, 1.25-4.14) compared with those who had normal iron markers and normal glucose.

**Conclusion:** The mortality risk of prediabetes is low. However, among individuals who have co-existing elevated iron markers, the risk rises substantially.

## STRENGTHS AND LIMITATIONS OF STUDY

- This study utilizes a nationally representative population-based cohort that allows generalization of results to the population of the United States.
- Mortality was left-censored to control for the potential misclassification of people who were very ill at baseline.
- Biomarkers for elevated iron and prediabetes were available only at baseline.
- We were able to only observe individuals for 12 years, which may be insufficient to adequately see an effect on mortality for prediabetes.

## INTRODUCTION

Prediabetes is defined by blood glucose concentrations that are higher than normal, but lower than established thresholds for diabetes.<sup>1</sup> Prediabetes is a high risk state for the development of not only diabetes, but also associated complications. Recent data have shown that in developed countries such as the United States and the United Kingdom more than a third of adults have prediabetes but the vast majority of these do not realize it.<sup>2-4</sup>

Several studies have shown that prediabetes is a mortality risk.<sup>5,6</sup> On the other hand, other studies have found that prediabetes is not a mortality risk.<sup>7-10</sup> Still other studies showed that the relationship was not very robust and was dependent on the measure of prediabetes.<sup>11</sup>

Three different meta-analyses of observational studies have concluded that elevated iron indices like serum ferritin and transferrin saturation (TS) are strongly associated with increased risk for developing diabetes.<sup>12-14</sup> In addition to increased risk of diabetes, elevated TS or elevated ferritin is associated with increased mortality in the general population.<sup>15-20</sup> Further, recent evidence suggests that among patients with diabetes, mortality risk increases in the presence of elevated ferritin or elevated TS.<sup>21,22</sup> We therefore hypothesize that the mortality risk of individuals with prediabetes will be increased in the presence of elevated iron markers.

Considering that only about 10% of the US population with prediabetes realize that they have prediabetes, a better understanding of the potential mortality risk is warranted. Consequently, the purpose of this study was to evaluate the association

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3 between prediabetes, elevated serum TS, elevated TS and elevated ferritin combined, and  
4 mortality in a large, nationally representative cohort.  
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## 7 8 **METHODS**

9 We conducted longitudinal analyses of the third National Health and Nutrition  
10 Examination Survey, 1988-1994 (NHANES III) linked to mortality data collected  
11 through the National Death Index. Mortality data were available through December 31,  
12 2006. The NHANES III survey provides population estimates of the United States and  
13 was conducted from October, 1988 through October, 1994. The NHANES III used  
14 complex, multi-stage, stratified, clustered samples of civilian, noninstitutionalized  
15 population. A total of 30,818 persons were examined in their homes or in mobile  
16 examination centers (MEC) which visited 89 communities across the United States. The  
17 health examination included collection of blood and urine specimens for the conduct of  
18 various laboratory analyses.  
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33 The NHANES III data merged with the National Death Index is a prospective  
34 cohort study that passively followed up on the participants in the NHANES III. The  
35 linked mortality file uses a probabilistic matching method.<sup>23</sup> We limited our study to  
36 individuals 40 years old and older at baseline, the time of their NHANES III interview.  
37 The National Death Index involves searching national databases containing information  
38 about mortality and causes of death. Mortality status was ascertained by computerized  
39 matching to national databases and evaluation of the resulting matches. Persons not found  
40 to be deceased were assumed alive for analytic purposes. All living survey participants  
41 had been observed for at least 146 months, and our survival analysis was carried out to  
42 December 31, 2006.  
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56 *Previously Diagnosed Diabetes*  
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3 The NHANES III assessed participants for diagnosed diabetes using the  
4 questions, “Have you ever been told by a doctor that you have diabetes or sugar  
5 diabetes?”, “Were you pregnant when you were told that you had diabetes?” and “Other  
6 than during pregnancy, has a doctor ever told you that you have diabetes or sugar  
7 diabetes?” We defined participants as having diagnosed diabetes if they answered “yes”  
8 to ever having been told they had diabetes, excluding pregnancy. Individuals with  
9 previously diagnosed diabetes were removed from the analysis. We also removed  
10 individuals with an HbA1c of 6.5% or greater, to account for undiagnosed diabetes.  
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### 22 *Prediabetes*

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24 We defined prediabetes among individuals without previously diagnosed diabetes  
25 using HbA1c ranges as specified by the American Diabetes Association, 5.7%–6.4% (39–  
26 46 mmol/mol).<sup>1</sup> This range has been shown in a meta-analysis to be predictive of  
27 progression to diabetes.<sup>18</sup> We excluded individuals with previously diagnosed diabetes  
28 because the current glycemic status of those patients may simply represent diabetes  
29 control. Prediabetes status was missing for 1,123 of the NHANES respondents over the  
30 age of 40.  
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### 40 *Transferrin Saturation*

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42 Serum iron and total iron-binding capacity (TIBC) were measured in serum, and  
43 calculated by dividing serum iron by TIBC and multiplying by 100. For the analyses, TS  
44 was categorized as: <50% and ≥50%. Despite the lack of universal agreement on the  
45 upper and lower limits of normal TS, this cut point has been used in several studies  
46 evaluating diabetes, TS and mortality.<sup>21,24</sup> Data was missing for transferrin saturation  
47 level for 1,288 of the NHANES respondents over the age of 40.  
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### *Serum Ferritin*

Serum ferritin was used as a measure of body iron stores and was measured using the QuantImmune Ferritin IRMA kit. Serum ferritin was categorized for the analyses as  $<898.80$  SI units and  $\geq 898.80$  SI units (400 ng/mL). Data was missing for serum ferritin level for 1,288 of the NHANES respondents over the age of 40.

### *Elevated Iron*

Individuals with both elevated serum ferritin and TS levels were considered to have elevated iron. Data was missing for elevated iron for 1,288 of the NHANES respondents over the age of 40.

### *Mortality*

Mortality was measured as all-cause mortality. Mortality status was ascertained solely by computerized matching to national databases and evaluation of the resulting matches. Mortality status was missing for 15 of the NHANES respondents over the age of 40.

### *Covariates*

Covariates used in our analyses included: age at baseline in the NHANES III, gender, race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, and other race), health insurance status, obesity (Body Mass Index computed in the exam of  $\geq 30$ ), previous diagnosis of a heart attack, previous diagnosis of a stroke, previous diagnosis of hypertension, previous diagnosis of hypercholesterolemia, previous diagnosis of cancer, family history of diabetes, family history of myocardial infarction before age 50, and current smoking status. Respondents were considered non-smokers if they reported



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3 smoking at least 100 cigarettes in their life or if they had smoked more than 100 cigarettes  
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5 and were not currently smoking.  
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### 8 *Analysis*

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10 In an effort to control for potential misclassification of persons who were very ill  
11 at baseline thereby affecting mortality risk of prediabetes, we left-censored the analysis to  
12 exclude any mortality events that occurred in the first three years following the  
13 individuals examination for the first three years of the cohort. For the analyses of  
14 mortality, we used sampling weights (specifically, the total MEC and Home examined  
15 weight) to calculate prevalence estimates for the civilian noninstitutionalized US  
16 population. Because of the complex sampling design of the survey, we performed  
17 statistical analyses using the statistical software package SUDAAN (Research Triangle  
18 Institute, Raleigh, NC), as recommended by the National Center for Health Statistics.  
19 Using SAS (Cary, NC), we computed Kaplan-Meier plots to show graphically the  
20 unadjusted relationship between all-cause mortality and prediabetes.  
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36 To accomplish our goals of examining a possible synergistic effect of having  
37 elevated iron with prediabetes we classified the population into 4 groups based upon  
38 prediabetes or normoglycemia and normal and elevated TS. We also classified the  
39 population into 4 groups based upon prediabetes or normoglycemia and normal and  
40 elevated iron based on levels of TS and ferritin.  
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48 We performed Cox proportional hazards analyses to measure the associations  
49 between all-cause mortality and prediabetes alone and for prediabetes controlling for all  
50 of the studied covariates using listwise deletion to account for missing data. In these  
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3 models, survival time was a continuous variable measured in 1-month increments from  
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5 the date of the exam.  
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8 We also performed unadjusted Cox proportional hazards analysis with all-cause  
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10 mortality for prediabetes in the 4 part variables with TS, ferritin and iron overload. We  
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12 then conducted analyses adjusting for the aforementioned covariates.  
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15 We evaluated the proportionality of the hazards through examination of the  
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17 Schoenfeld residuals.  
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## 19 20 RESULTS

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22 A total of 8,041 (unweighted) individuals were over 40 years old and had HbA1c  
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24 levels under 6.4%. Baseline characteristics for the sample are shown in Table 1. Table 1  
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26 indicates that 23.0% of the weighted sample had prediabetes, less than 7.0% of the  
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28 sample exhibited elevated serum ferritin, and less than 4.0% had elevated TS. Elevated  
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30 iron was indicated in 1.4% of the sample.  
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34 Table 2 shows the results of unadjusted and adjusted Cox proportional hazards  
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36 models for prediabetes. The Kaplan Meier curve of the survival and prediabetes over the  
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38 length of the time under observation is shown in Fig 1.  
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41 Table 3 presents results of the analyses combining prediabetes with iron markers.  
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43 In models that examined the impact of a prediabetes state combined with markers of low  
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45 iron, the hazard ratios were similar to that of prediabetes alone. However, when  
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47 combined with prediabetes, there was an increased mortality risk among individuals with  
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49 TS  $\geq 50\%$ , and even higher when individuals had TS  $> 50\%$  and serum ferritin  $> 898.80$  SI  
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51 units (400 ng/mL). Figure 2 represents the relationship of survival of the four groups over  
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53 the 12 years under observation. Individuals with prediabetes in the presence of elevated  
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3 iron have lower survival probabilities than other groups. An examination of the  
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5 Schoenfeld residuals suggested proportionality of hazards and appropriateness of the  
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7 statistical model for these analyses.  
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## 10 11 **DISCUSSION**

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13 The results of this study in a nationally representative cohort that followed  
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15 individuals for 12 years confirm that the mortality risk of prediabetes is probably low.  
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17 This is not unexpected based on the mixed results from previous studies, several of which  
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19 found either no future mortality risk or risk that was not robust across measures.  
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21 However, we found that the presence of markers of elevated iron is associated with  
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23 increased mortality risk of individuals with prediabetes. Among individuals with normal  
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25 iron levels, those with prediabetes had low mortality risk levels similar to the adjusted  
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27 risk of prediabetes alone. On the other hand, in adjusted survival analyses, individuals  
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29 with prediabetes who also had elevated iron levels had more than twice the mortality risk.  
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31 These findings extend previous work on iron markers and diabetes to the previously  
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33 uninvestigated area of prediabetes.  
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40 These results suggest that additional stratification of individuals with prediabetes  
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42 on the basis of iron markers would be useful to identify those with higher risk and who  
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44 might benefit from iron lowering therapies. Previous data has indicated that elevated iron  
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46 markers are associated with the development of diabetes and that among individuals with  
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48 diabetes the co-occurrence of elevated TS increases those patients' mortality risk. Early  
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50 identification of individuals with both conditions (prediabetes, elevated iron) may help in  
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52 both slowing the development of diabetes as well as decreasing mortality risk. It is  
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54 important for early identification of these individuals because much like individuals with  
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3 prediabetes, the vast majority of individuals with elevated iron do not know it.<sup>25</sup> These  
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5 individuals need to be identified to mitigate the increased risk posed by elevated iron in  
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7 combination with prediabetes. Such individuals would be targets for intensive  
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9 interventions to reduce risk, including typical lifestyle interventions shown to help avoid  
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11 the onset of diabetes in people at high risk.<sup>26</sup> Although more research is needed into the  
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13 ability of interventions on iron in prediabetes to affect development of diabetes and  
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15 mortality risk, some data suggest that reduction of TS improves HbA1c and glucose  
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17 control.<sup>27</sup>  
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22 These associations of TS and ferritin with mortality in the context of prediabetes  
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24 are not surprising especially if elevations of these parameters are interpreted in light of  
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26 current understanding of iron toxicity.<sup>28</sup> Iron, whether absorbed as iron salts or in dietary  
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28 heme is processed by enterocytes and released into the plasma where it is transported in a  
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30 non-reactive state bound to transferrin. Iron that is bound to transferrin is in the Fe<sup>+3</sup>  
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32 state and is not reactive and, therefore, not toxic. However, when TS is above 40 to 50%,  
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34 free iron or so-called non-transferrin-bound iron (NTBI) is released into the plasma as the  
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36 buffering ability of transferrin is exceeded.<sup>29</sup> Labile plasma iron (LPI) is a highly  
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38 reactive subspecies of NTBI that interacts with hydrogen peroxide through Fenton  
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40 chemistry to form the extremely powerful oxidants, hydroxyl radical and singlet oxygen.  
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42 These are the free radicals that ultimately directly damage protein and DNA. Perhaps  
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44 more importantly, NTBI/LPI species are able to enter cells via ion channels. These  
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46 channels, unlike the transferrin receptor, are not regulated so this reactive iron freely  
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48 enters the cytoplasm of the pancreas, pituitary, and heart.  
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The current results suggest that exposure to excessive free iron is dangerous in the context of prediabetes. Furthermore, elevated ferritin and TS predict poor diabetes control and phlebotomy to reduce iron even over short periods of time improve HbA1c in parallel with changes in TS, even though ferritin is not changed,<sup>27</sup> putting further emphasis on the fact that NTBI/LPI reflected by TS is the proximal cause of the toxicity. Reduction in TS by phlebotomy has been shown to improve measures of diabetic control.<sup>27</sup> Furthermore, correction of severe iron overload can significantly improve glucose tolerance.<sup>30</sup> Thus, the finding that a baseline measure of high TS as point measure of toxic free iron plus elevation of ferritin, evidence of elevated cytosolic iron over a longer period of time, predicts increased risk of mortality among individuals with prediabetes supports the premise that toxic free iron is a health risk.

This study has several limitations. First, although we have a nationally representative, population-based cohort followed through the National Death Index, the biomarkers are measured only at baseline. There is the possibility that either the hyperglycemia or elevated iron measures were identified and interventions were implemented to lower these biomarkers. If that were the case and a substantial number of individuals did drop their levels due to interventions thereby decreasing the potential mortality risk, the observed adjusted risk of 2.28 for individuals elevated at baseline is even more concerning. Second, we were only able to follow these individuals for a little more than 12 years. It is possible that this time frame may have been too short to adequately see an effect for a biomarker like prediabetes. However, we did censor the first three years of mortality so that any deaths in that time frame would not be attributed

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3 to prediabetes. The model still found a substantial mortality risk for the prediabetes plus  
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5 iron markers in this length of time.  
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8 In conclusion, this study representative of the population of the United States  
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10 helps to clarify the current evidence on the mortality risk of prediabetes and provides  
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12 further support for the role of elevated iron markers in health risk. Future screening and  
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14 intervention programs for prediabetes may benefit from additional strategies to recognize  
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16 and treat iron elevations.  
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## CONTRIBUTION STATEMENT

AGM: conception, design, analysis, and drafting of manuscript

RJT: design, analysis, and drafting of manuscript

TDC: conception, design, and drafting of manuscript

RB: design and analysis

## COMPETING INTERESTS

The authors report no competing interests.

## DATA SHARING STATEMENT

Data from this study are publicly available on the Internet through the National Center for Health Statistics.

**REFERENCES**

1. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 2012;35:S64–S71.
2. Bullard KM, Saydah SH, Imperatore G, et al. Secular changes in U.S. Prediabetes prevalence defined by hemoglobin A1c and fasting plasma glucose: National Health and Nutrition Examination Surveys, 1999-2010. *Diabetes Care* 2013;36:2286–93.
3. Mainous AG 3rd, Tanner RJ, Baker R, et al. Prevalence of prediabetes in England from 2003 to 2011: population-based, cross-sectional study. *BMJ Open* 2014 Jun 9;4(6):e005002. doi: 10.1136/bmjopen-2014-005002. Accessed 29 June, 2014.
4. Centers for Disease Control and Prevention (CDC). Awareness of prediabetes--United States, 2005-2010. *MMWR Morb Mortal Wkly Rep*. 2013 Mar 22;62:209-12.
5. de Vegt F, Dekker JM, Ruhé HG, et al. Hyperglycaemia is associated with all-cause and cardiovascular mortality in the Hoorn population: the Hoorn Study. *Diabetologia* 1999;42:926–31.
6. Fuller JH, Shipley MJ, Rose G, et al. Coronary-heart-disease risk and impaired glucose tolerance. The Whitehall study. *Lancet* 1980;1:1373–76.
7. Valdés S, Botas P, Delgado E, Cadórniga FD. Mortality risk in Spanish adults with diagnosed diabetes, undiagnosed diabetes, or pre-diabetes. The asturias study 1998-2004. *Rev Esp Cardiol (Engl Ed)* 2009;62:528–34.
8. Deedwania P, Patel K, Fonarow GC, et al. Prediabetes is not an independent risk factor for incident heart failure, other cardiovascular events or mortality in older adults: findings from a population-based cohort study. *Int J Cardiol* 2013;168:3616–22.



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9. Kowall B, Rathmann W, Heier M, et al. Categories of glucose tolerance and continuous glycemic measures and mortality. *Eur J Epidemiol* 2011;26:637–45.
10. Zhou XH, Qiao Q, Zethelius B, et al. Diabetes, prediabetes and cancer mortality. *Diabetologia* 2010;53:1867–76.
11. Barr EL, Zimmet PZ, Welborn TA, et al. Risk of cardiovascular and all-cause mortality in individuals with diabetes mellitus, impaired fasting glucose, and impaired glucose tolerance: the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab). *Circulation* 2007;116:151–57.
12. Orban E, Schwab S, Thorand B, Huth C. Association of iron indices and type 2 diabetes: a meta-analysis of observational studies. *Diabetes Metab Res Rev* 2014;30:372–94.
13. Bao W, Rong Y, Rong S, Liu L. Dietary iron intake, body iron stores, and the risk of type 2 diabetes: a systematic review and meta-analysis. *BMC Med* 2012;10:119. doi: 10.1186/1741-7015-10-119. Accessed 8 July, 2014.
14. Ellervik C, Mandrup-Poulsen T, Tybjærg-Hansen A, Nordestgaard BG. Total and cause-specific mortality by elevated transferrin saturation and hemochromatosis genotype in individuals with diabetes: two general population studies. *Diabetes Care* 2014;37:444–52.
15. Ellervik C, Tybjaerg-Hansen A, Nordestgaard BG. Total mortality by transferrin saturation levels: Two general population studies and a meta analysis. *Clin Chem* 2011;57:459–466.
16. Kim KS, Son HG, Hong NS, Lee DH. Associations of serum ferritin and transferrin % saturation with all-cause, cancer, and cardiovascular disease mortality: Third National

1  
2  
3 Health and Nutrition Examination Survey follow-up study. *J Prev Med Public Health*  
4  
5 2012;45:196-203.  
6

7  
8 17. Mainous AG 3rd, Gill JM, Carek PJ. Elevated transferrin saturation and mortality.  
9  
10 *Ann Fam Med* 2004;2:133–138.  
11

12  
13 18. Mainous AG 3rd, Wells B, Carek PJ, et al. The mortality risk of elevated serum  
14  
15 transferrin saturation and consumption of dietary iron. *Ann Fam Med* 2004;2:139-44.  
16

17  
18 19. Stack AG, Mutwali AI, Nguyen HT, et al. Transferrin saturation ratio and risk of total  
19  
20 and cardiovascular mortality in the general population. *QJM*. 2014;107:623-33.  
21

22  
23 20. Wells BJ, Mainous AG 3rd, King DE, et al. The combined effect of transferrin  
24  
25 saturation and low density lipoprotein on mortality. *Fam Med* 2004;36:324-9.  
26

27  
28 21. Ponikowska B, Suchocki T, Paleczny B, et al. Iron status and survival in diabetic  
29  
30 patients with coronary artery disease. *Diabetes Care* 2013;36:4147–56.  
31

32  
33 22. Morris DH, Khunti K, Achana F, et al. Progression rates from HbA(1c) 6.0-6.4% and  
34  
35 other prediabetes definitions to type 2 diabetes: a meta-analysis. *Diabetologia*  
36  
37 2013;56:1489–93.  
38

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40 23. National Center for Health Statistics. Office of Analysis and Epidemiology, NCHS  
41  
42 2011 Linked Mortality Files Matching Methodology, September, 2013. Hyattsville,  
43  
44 Maryland.  
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46 [http://www.cdc.gov/nchs/data/datalinkage/2011\\_linked\\_mortality\\_file\\_matching\\_method](http://www.cdc.gov/nchs/data/datalinkage/2011_linked_mortality_file_matching_methodology.pdf)  
47  
48 [ology.pdf](http://www.cdc.gov/nchs/data/datalinkage/2011_linked_mortality_file_matching_methodology.pdf). Accessed 20 August, 2014.  
49

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51 24. Ellervik C, Andersen HU, Tybjærg-Hansen A, et al. Total mortality by elevated  
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53 transferrin saturation in patients with diabetes. *Diabetes Care* 2013;36:2646–54.  
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25. Mainous AG 3rd, Wright RU, Hulihan MM, et al. Elevated transferrin saturation, health-related quality of life and telomere length. *Biometals* 2014;27:135–41
26. Portero McLellan KC, Wyne K, Villagomez ET, Hsueh WA. Therapeutic interventions to reduce the risk of progression from prediabetes to type 2 diabetes mellitus. *Ther Clin Risk Manag* 2014;10:173–88.
27. Fernández-Real JM, Peñarroja G, Castro A, García-Bragado F, Hernández-Aguado I, Ricart W. Blood letting in high-ferritin type 2 diabetes: effects on insulin sensitivity and beta-cell function. *Diabetes* 2002;51:1000–4.
28. Hansen JB, Moen IW, Mandrup-Poulsen T. Iron: the hard player in diabetes pathophysiology. *Acta Physiol* 2014;210:717–32.
29. Coates TD. Physiology and pathophysiology of iron in hemoglobin-associated diseases. *Free Radic Biol Med* 2014;72C:23–40.
30. Farmaki K, Tzoumari I, Pappa C, et al. Normalisation of total body iron load with very intensive combined chelation reverses cardiac and endocrine complications of thalassaemia major. *Br J Haematol* 2010;148:466–75.

Table 1: Baseline Characteristics of the Cohort

	<b>All Respondents (%)</b>
<b>Unweighted Sample Size</b>	8,041
<b>Weighted Sample Size</b>	81,152,997
<b>Age, years</b>	
40-54	49.7
55-69	31.2
70+	19.2
<b>Sex, male</b>	46.3
<b>Race/Ethnicity</b>	
Non-Hispanic White	82.1
Non-Hispanic Black	8.3
Hispanic	6.6
Asian/Other	2.9
<b>Has health insurance</b>	93.5
<b>Obese</b>	23.5
<b>Current Smoker, %</b>	23.5
<b>Has diagnosed hypertension, %</b>	31.3
<b>Ever had heart attack, %</b>	2.8
<b>Ever had stroke, %</b>	5.3
<b>Ever had cancer, %</b>	12.3
<b>Relative with diabetes, %</b>	41.0
<b>Relative with heart attack before age 50, %</b>	15.4
<b>High Transferrin Saturation, %</b>	3.9
<b>High Serum Ferritin, %</b>	6.6
<b>Elevated Iron, %</b>	1.4
<b>Prediabetes, %</b>	23.0
<b>Assumed deceased, %</b>	30.7

Table 2: Unadjusted and Adjusted Hazard Ratios from Cox Regression for Mortality Risk of Individuals with Prediabetes

	<b>Hazard Ratio</b>	<b>95% Confidence Interval</b>
<b>Prediabetes</b>		
<b>Unadjusted Model</b>		
Prediabetes	1.14	1.10-1.18
Normoglycemia	1.0	
<b>Adjusted Model<sup>a</sup></b>		
Prediabetes	1.04	1.00-1.09
Normoglycemia	1.0	

<sup>a</sup>Adjusted for: age, gender, race/ethnicity, health insurance status, obesity, current smoking status, diagnosed heart attack, diagnosed stroke, diagnosed hypertension, diagnosed high cholesterol, diagnosis of cancer, family history of diabetes, and family history of early heart attack.

Table 3: Unadjusted and Adjusted Hazard Ratios from Cox Regression for Mortality Risk of Individuals with Prediabetes and Iron Markers

<b>Unadjusted Model</b>	<b>Hazard Ratio</b>	<b>95% Confidence Interval</b>
Normal TS/Normoglycemia	1.0	
Normal TS/Prediabetes	1.14	1.10-1.18
High TS/Normoglycemia	1.01	0.94-1.09
High TS/Prediabetes	1.39	1.09-1.77
<b>Adjusted Model<sup>a</sup></b>		
Normal TS/Normoglycemia	1.0	
Normal TS/Prediabetes	1.04	1.00-1.08
High TS/Normoglycemia	1.05	0.93-1.18
High TS/Prediabetes	1.56	1.12-2.17
<b>Unadjusted Model</b>		
Normal TS+Ferritin/Normoglycemia	1.0	
Normal TS+Ferritin Iron/Prediabetes	1.14	1.10-1.18
Elevated TS+Ferritin/Normoglycemia	1.1	0.93-1.29
Elevated TS+Ferritin/Prediabetes	1.87	1.18-2.95
<b>Adjusted Model<sup>a</sup></b>		
Normal TS+Ferritin/Normoglycemia	1.0	
Normal TS+Ferritin Iron/Prediabetes	1.04	1.00-1.08
Elevated TS+Ferritin/Normoglycemia	1.20	0.94-1.53
Elevated TS+Ferritin/Prediabetes	2.28	1.25-4.14

<sup>a</sup>Adjusted for: age, gender, race/ethnicity, health insurance status, obesity, current smoking status, diagnosed heart attack, diagnosed stroke, diagnosed hypertension,

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3 diagnosed high cholesterol, diagnosis of cancer, family history of diabetes, and family  
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For peer review only

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3 Figure 1. Kaplan Meier curve of survival among individuals with prediabetes or normal  
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10 --- Prediabetes

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15 Figure 2. Kaplan Meier curve of survival among individuals with prediabetes and  
16  
17 elevated transferrin saturation and elevated ferritin

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19 — Normoglycemia and Elevated Iron

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21 --- Normoglycemia and Normal Iron

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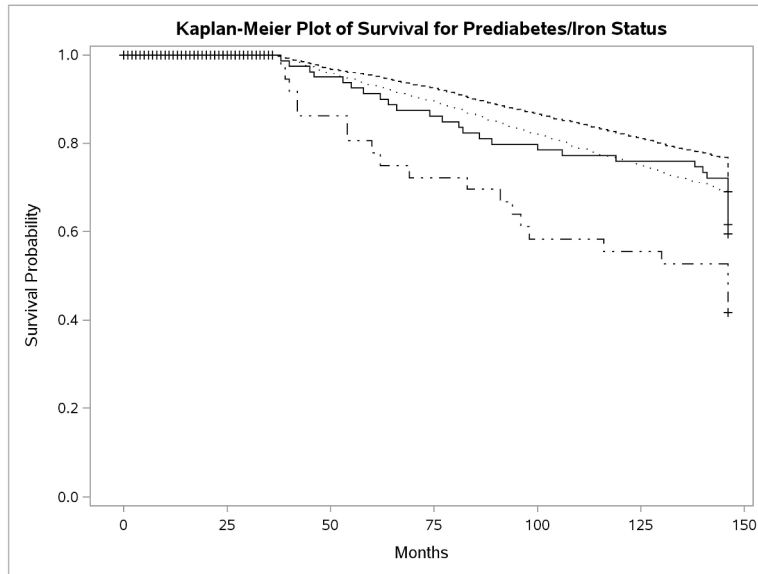


Figure 2. Kaplan Meier curve of survival among individuals with prediabetes and elevated transferrin saturation and elevated ferritin

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- Normoglycemia and Normal Iron
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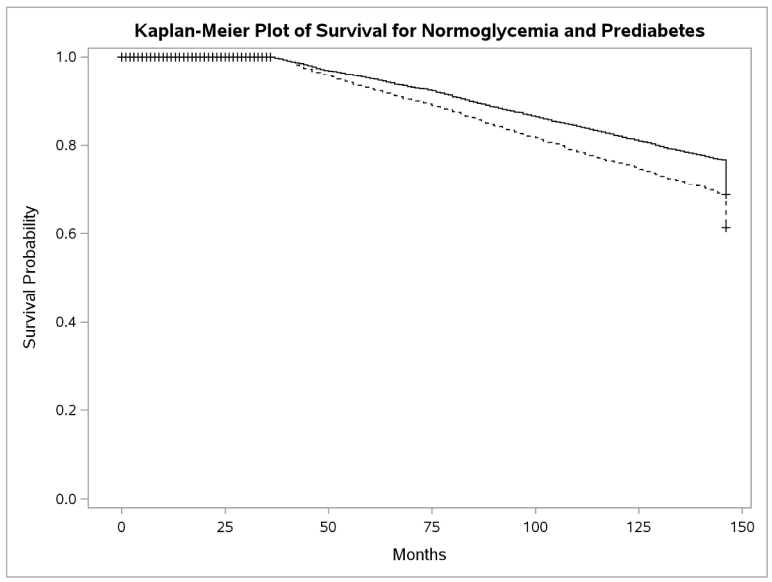


Figure 1. Kaplan Meier curve of survival among individuals with prediabetes or normal glycaemic levels.  
 ----- Normoglycemia  
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267x350mm (300 x 300 DPI)

## STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page number
<b>Title and abstract</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
<b>Introduction</b>			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4-5
<b>Methods</b>			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	5
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-8
Bias	9	Describe any efforts to address potential sources of bias	9
Study size	10	Explain how the study size was arrived at	5-8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	8-9
		(b) Describe any methods used to examine subgroups and interactions	8
		(c) Explain how missing data were addressed	8
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	NA
		(e) Describe any sensitivity analyses	NA

Continued on next page

<b>Results</b>			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5, 9
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9, 19
		(b) Indicate number of participants with missing data for each variable of interest	6-7
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	5
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	19
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-10 20- 22
		(b) Report category boundaries when continuous variables were categorized	NA
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	10- 11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12- 13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).

# BMJ Open

## Prediabetes, Elevated Iron, and All-Cause Mortality: A Cohort Study

Journal:	<i>BMJ Open</i>
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<b>Primary Subject Heading</b>:	Diabetes and endocrinology
Secondary Subject Heading:	Haematology (incl blood transfusion)
Keywords:	General diabetes < DIABETES & ENDOCRINOLOGY, HAEMATOLOGY, DIABETES & ENDOCRINOLOGY

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Manuscripts

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3 **Prediabetes, Elevated Iron, and All-Cause Mortality: A Cohort Study**  
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5 Running Title: Prediabetes, Elevated Iron, and All-Cause Mortality  
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8 Arch G. Mainous III, Rebecca J. Tanner, Thomas D. Coates, Richard Baker  
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56 **Key Words:** prediabetes, transferrin saturation, ferritin, NHANES, Mortality  
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## ABSTRACT

**Objectives:** Data have indicated low to nonexistent increased mortality risk for individuals with prediabetes, but it is unclear if the risk is increased when the patient has elevated iron markers. Our purpose was to examine the mortality risk among adults with prediabetes in the context of co-existing elevated transferrin saturation (TS) or serum ferritin.

**Setting:** Data collected by the third National Health and Nutrition Examination Survey 1988-1994 (NHANES III) in the United States and by the National Center for Health Statistics for the National Death Index from 1988-2006.

**Participants:** Individuals age 40 and older who participated in the NHANES and provided a blood sample.

**Primary Outcome Variable:** Mortality was measured as all-cause mortality.

**Results:** Adjusted analyses show that prediabetes has a small increased mortality risk (HR =1.04; 95% CI, 1.00-1.08). Persons who had prediabetes and elevated serum ferritin had an increased hazard ratio for death (HR=1.14; 95% CI, 1.04-1.24) compared with those who had normal ferritin and normal glucose. Among persons with prediabetes who had elevated TS, they had an increased mortality risk (HR=1.86; 95% CI, 1.05-3.29) compared with those with normal TS levels and normal glucose. **Conclusion:** The mortality risk of prediabetes is low. However, among individuals who have co-existing elevated iron markers, particularly transferrin saturation, the risk rises substantially.

## STRENGTHS AND LIMITATIONS OF STUDY

- This study utilizes a nationally representative population-based cohort that allows generalization of results to the population of the United States.
- Mortality was left-censored to control for the potential misclassification of people who were very ill at baseline.
- Biomarkers for elevated iron and prediabetes were available only at baseline.
- We were able to only observe individuals for 12 years, which may be insufficient to adequately see an effect on mortality for prediabetes.



## INTRODUCTION

Prediabetes is defined by blood glucose concentrations that are higher than normal, but lower than established thresholds for diabetes.<sup>1</sup> Prediabetes is a high risk state for the development of not only diabetes, but also associated complications. Recent data have shown that in developed countries such as the United States and the United Kingdom more than a third of adults have prediabetes but the vast majority of these do not realize it.<sup>2-4</sup>

Several studies have shown that prediabetes is a mortality risk.<sup>5,6</sup> On the other hand, other studies have found that prediabetes is not a mortality risk.<sup>7-10</sup> Still other studies showed that the relationship was not very robust and was dependent on the measure of prediabetes.<sup>11</sup>

Three different meta-analyses of observational studies have concluded that elevated iron indices like serum ferritin and transferrin saturation (TS) are strongly associated with increased risk for developing diabetes.<sup>12-14</sup> Some evidence exists to indicate that pancreatic beta cells are killed in the presence of iron.<sup>15</sup> In addition to increased risk of diabetes, elevated TS or elevated ferritin is associated with increased mortality in the general population.<sup>16-22</sup> Further, recent evidence suggests that among patients with diabetes, mortality risk increases in the presence of elevated ferritin or elevated TS.<sup>23,24</sup> We therefore hypothesize that the mortality risk of individuals with prediabetes will be increased in the presence of elevated iron markers.

Considering that only about 10% of the US population with prediabetes realize that they have prediabetes, a better understanding of the potential mortality risk is warranted. Consequently, the purpose of this study was to evaluate the association

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3 between prediabetes, elevated serum ferritin, elevated TS and mortality in a large,  
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5 nationally representative cohort.  
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## 8 **METHODS**

9 We conducted longitudinal analyses of the third National Health and Nutrition  
10 Examination Survey, 1988-1994 (NHANES III) linked to mortality data collected  
11 through the National Death Index. Mortality data were available through December 31,  
12 2006. The NHANES III survey provides population estimates of the United States and  
13 was conducted from October, 1988 through October, 1994. The NHANES III used  
14 complex, multi-stage, stratified, clustered samples of civilian, noninstitutionalized  
15 population. Of the 39,695 individuals eligible to participate, a total of 30,818 persons  
16 were examined in their homes or in mobile examination centers (MEC) which visited 89  
17 communities across the United States (a participation rate of 77.6%). The health  
18 examination included collection of blood and urine specimens for the conduct of various  
19 laboratory analyses. The NHANES provides preexisting de-identified public use data  
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37 The NHANES III data merged with the National Death Index is a prospective  
38 cohort study that passively followed up on the participants in the NHANES III. The  
39 linked mortality file uses a probabilistic matching method.<sup>25</sup> We limited our study to  
40 individuals 40 years old and older at baseline, the time of their NHANES III interview.  
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42 The National Death Index involves searching national databases containing information  
43 about mortality and causes of death. Mortality status was ascertained by computerized  
44 matching to national databases and evaluation of the resulting matches. Persons not found  
45 to be deceased were assumed alive for analytic purposes. All living survey participants  
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3 had been observed for 146 months, and our survival analysis was carried out to  
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### 7 8 *Previously Diagnosed Diabetes*

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10 The NHANES III assessed participants for diagnosed diabetes using the  
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12 questions, “Have you ever been told by a doctor that you have diabetes or sugar  
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14 diabetes?”, “Were you pregnant when you were told that you had diabetes?” and “Other  
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16 than during pregnancy, has a doctor ever told you that you have diabetes or sugar  
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18 diabetes?” We defined participants as having diagnosed diabetes if they answered “yes”  
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20 to ever having been told they had diabetes, excluding pregnancy. Individuals with  
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22 previously diagnosed diabetes were removed from the analysis. We also removed  
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24 individuals with an HbA1c of 6.5% or greater, to account for undiagnosed diabetes.  
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### 29 30 *Normoglycemia & Prediabetes*

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32 We defined normoglycemia as an HbA1c level between 4.0%--5.6% (20-38 mmol/mol).  
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34 To control for any potential effect of low HbA1c, we also removed individuals with an  
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36 HbA1c below 4.0% (20 mmol/mol), a level associated with increased all-cause mortality  
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38 in adults without diabetes.<sup>26</sup>

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41 We defined prediabetes among individuals without previously diagnosed diabetes  
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43 using HbA1c ranges as specified by the American Diabetes Association, 5.7%–6.4% (39-  
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45 46 mmol/mol).<sup>1</sup> This range has been shown in a meta-analysis to be predictive of  
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47 progression to diabetes.<sup>19</sup> We excluded individuals with previously diagnosed diabetes  
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49 because the current glycemic status of those patients may simply represent diabetes  
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51 control. Prediabetes status was missing for 1,637 of the NHANES respondents over the  
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60 age of 40.

### *Transferrin Saturation*

Serum iron and total iron-binding capacity (TIBC) were measured in serum, and calculated by dividing serum iron by TIBC and multiplying by 100. For the analyses, elevated transferrin saturation was categorized as TS  $\geq 50\%$ . Individuals with TS below 25% were removed from the analysis, as low TS has been linked to increased risk of mortality.<sup>23</sup> Despite the lack of universal agreement on the upper and lower limits of normal TS, these cut points has been used in several studies evaluating diabetes, TS and mortality.<sup>23,27</sup> Data were missing for transferrin saturation level for 536 of the NHANES respondents over the age of 40.

### *Serum Ferritin*

Serum ferritin was used as a measure of body iron stores and was measured using the QuantImmune Ferritin IRMA kit. Serum ferritin was categorized for the analyses as elevated if it was  $\geq 674.1$  pmol/l (300 ng/mL) for males and  $\geq 449.4$  pmol/l (200 ng/mL) for females.<sup>28</sup> Individuals with serum ferritin below 56.175 pmol/l (25 ng/mL) were removed from the analysis, as low ferritin has been linked to an increased risk of mortality.<sup>23</sup> Data were missing for serum ferritin level for 539 of the NHANES respondents over the age of 40.

### *Mortality*

Mortality was measured as all-cause mortality. Mortality status was ascertained solely by computerized matching to national databases and evaluation of the resulting matches.

### *Covariates*

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Covariates used in our analyses included: age at baseline in the NHANES III, gender, race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, and other race), health insurance status, obesity (Body Mass Index computed in the exam of  $\geq 30$ ), previous diagnosis of a heart attack, previous diagnosis of a stroke, previous diagnosis of hypertension, previous diagnosis of hypercholesterolemia, previous diagnosis of cancer, family history of diabetes, family history of myocardial infarction before age 50, and current smoking status. Respondents were considered non-smokers if they reported smoking at least 100 cigarettes in their life or if they had smoked more than 100 cigarettes and were not currently smoking.

In the analysis of serum ferritin, we also controlled for C-reactive protein. Ferritin is an acute phase reactant as well as an indicator of iron stores and as such may indicate inflammation. Consequently, we controlled for inflammation by adjusting for C-reactive protein. C-reactive protein was considered elevated at levels above 3.0 mg/l.<sup>29</sup>

### *Analysis*

In an effort to control for potential misclassification of persons who were very ill at baseline thereby affecting mortality risk of prediabetes, we left-censored the analysis to exclude any mortality events that occurred in the first three years following the individuals examination for the first three years of the cohort. 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. This strategy of basing the analyses on population estimates is a characteristic that makes the NHANES different from many other cohort designs that do not use weighted population estimates. However, this strategy does provide national

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3 generalizability. Because of the complex sampling design of the survey, we performed  
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5 statistical analyses using the statistical software package SUDAAN (Research Triangle  
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7 Institute, Raleigh, NC), as recommended by the National Center for Health Statistics  
8  
9 (NCHS). Using SAS (Cary, NC), we computed Kaplan-Meier plots to show graphically  
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11 the unadjusted relationship between all-cause mortality and prediabetes.  
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15 All analyses were based on the population estimates and we followed the NCHS  
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17 recommendations for assessing the reliability of estimates in the context of a limited  
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19 sample size. If the standard error of an estimate was greater than 30% of an estimate it  
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21 would be considered unreliable All estimates met the criteria for reliability.  
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25 To accomplish our goals of examining a possible synergistic effect of having  
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27 elevated iron with prediabetes we classified the population into 4 groups based upon  
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29 prediabetes or normoglycemia and normal and elevated TS. We also classified the  
30  
31 population into 4 groups based upon prediabetes or normoglycemia and normal and  
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33 elevated iron based on levels of TS and ferritin.  
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37 We performed Cox proportional hazards analyses to measure the associations  
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39 between all-cause mortality and prediabetes controlling for all of the studied covariates  
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41 using listwise deletion to account for missing data. In these models, survival time was a  
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43 continuous variable measured in 1-month increments from the date of the exam.  
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47 We also performed adjusted Cox proportional hazards analysis with all-cause  
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49 mortality for prediabetes in the 4 part variables with TS adjusting for the aforementioned  
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51 covariates. For the adjusted Cox proportional hazards analysis with ferritin we adjusted  
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53 for the aforementioned covariates and also C-reactive protein.  
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3 We evaluated the proportionality of the hazards through examination of the  
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5 Schoenfeld residuals.  
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## 7 8 **RESULTS** 9

10 A total of 8,003 (unweighted) individuals were over 40 years old and had HbA1c  
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12 levels between 4.0 and 6.4%. Baseline characteristics for the sample are shown in Table

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15 1. Table 1 indicates that 23.2% of the weighted sample had prediabetes, 15.6% of the  
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17 sample exhibited elevated serum ferritin, and 3.3% had elevated TS.  
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19  
20 Of the respondents that had prediabetes, 38.8% died within 12 years, compared to  
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22 23.4% of respondents with normal HbA1c levels. Among individuals with normal TS  
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24 and normoglycemia, 23.1% died, compared to 23.7% of those with elevated TS, 37.5% of  
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26 those with normal TS and prediabetes, and 44.7% of those with elevated TS and  
27  
28 prediabetes. Among individuals with normal ferritin and normoglycemia, 34.3% died,  
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30 compared with 38.8% of those with normal ferritin and prediabetes, 29.2% of those with  
31  
32 elevated ferritin and normoglycemia, and 38.8% of those with elevated ferritin and  
33  
34 prediabetes.  
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38  
39 Table 2 shows the results the adjusted Cox proportional hazards model for  
40  
41 prediabetes. Table 2 indicates prediabetes alone has a small increased mortality risk. The  
42  
43 Kaplan Meier curve of the survival and prediabetes over the length of the time under  
44  
45 observation is shown in Fig 1.  
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48  
49 Table 3 presents results of the analyses combining prediabetes with iron markers.  
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51 In models that examined the impact of a prediabetes state combined with markers of low  
52  
53 iron, the hazard ratios were similar to that of prediabetes alone. However, when  
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55 combined with prediabetes, there was an increased mortality risk among individuals with  
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3 TS  $\geq 50$ , as well as with individuals who had increased ferritin. The risk was most  
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5 increased when individuals had elevated ferritin and elevated transferrin saturation  
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7 together. Figure 2 represents the relationship of survival of the four groups over the 12  
8  
9 years under observation. Individuals with prediabetes in the presence of elevated iron  
10  
11 have lower survival probabilities than other groups. An examination of the Schoenfeld  
12  
13 residuals suggested proportionality of hazards and appropriateness of the statistical model  
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15 for these analyses.  
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## 20 21 **DISCUSSION**

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23 The results of this study in a nationally representative cohort that followed  
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25 individuals for 12 years confirm that the mortality risk of prediabetes is probably low.  
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27 This is not unexpected based on the mixed results from previous studies, several of which  
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29 found either no future mortality risk or risk that was not robust across measures.  
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31 However, we found that the presence of transferrin saturation and serum ferritin is  
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33 associated with increased mortality risk of individuals with prediabetes. Among  
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35 individuals with normal iron levels, those with prediabetes had low mortality risk levels  
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37 similar to the adjusted risk of prediabetes alone. On the other hand, in adjusted survival  
38  
39 analyses, individuals with prediabetes who also had elevated transferrin saturation had  
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41 substantially increased mortality risk. These findings extend previous work on iron  
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43 markers and diabetes to the previously uninvestigated area of prediabetes.  
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49 These results suggest that additional stratification of individuals with prediabetes  
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51 on the basis of iron markers would be useful to identify those with higher risk and who  
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53 might benefit from iron lowering therapies. Previous data has indicated that elevated iron  
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55 markers are associated with the development of diabetes and that among individuals with  
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3 diabetes the co-occurrence of elevated TS increases those patients' mortality risk. Early  
4 identification of individuals with both conditions (prediabetes, elevated iron) may help in  
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6 both slowing the development of diabetes as well as decreasing mortality risk. It is  
7  
8 important for early identification of these individuals because much like individuals with  
9  
10 prediabetes, the vast majority of individuals with elevated iron do not know it.<sup>30</sup> These  
11  
12 individuals need to be identified to mitigate the increased risk posed by elevated iron in  
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14 combination with prediabetes. Such individuals would be targets for intensive  
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16 interventions to reduce risk, including typical lifestyle interventions shown to help avoid  
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18 the onset of diabetes in people at high risk.<sup>31</sup> Although more research is needed into the  
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20 ability of interventions on iron in prediabetes to affect development of diabetes and  
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22 mortality risk, some data suggest that reduction of TS improves HbA1c and glucose  
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24 control.<sup>32</sup>

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32 These associations of TS and ferritin with mortality in the context of prediabetes  
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34 are not surprising especially if elevations of these parameters are interpreted in light of  
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36 current understanding of iron toxicity.<sup>33</sup> Iron, whether absorbed as iron salts or in dietary  
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38 heme is processed by enterocytes and released into the plasma where it is transported in a  
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40 non-reactive state bound to transferrin. Iron that is bound to transferrin is in the  $\text{Fe}^{+3}$   
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42 state and is not reactive and, therefore, not toxic. However, when TS is above 40 to 50%,  
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44 free iron or so-called non-transferrin-bound iron (NTBI) is released into the plasma as the  
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46 buffering ability of transferrin is exceeded.<sup>34</sup> Labile plasma iron (LPI) is a highly  
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48 reactive subspecies of NTBI that interacts with hydrogen peroxide through Fenton  
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50 chemistry to form the extremely powerful oxidants, hydroxyl radical and singlet oxygen.  
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52 These are the free radicals that ultimately directly damage protein and DNA. Perhaps  
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3 more importantly, NTBI/LPI species are able to enter cells via ion channels. These  
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5 channels, unlike the transferrin receptor, are not regulated so this reactive iron freely  
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7 enters the cytoplasm of the pancreas, pituitary, and heart.  
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10 The current results suggest that exposure to excessive free iron is dangerous in the  
11  
12 context of prediabetes. Furthermore, elevated ferritin and TS predict poor diabetes  
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14 control and phlebotomy to reduce iron even over short periods of time improve HbA1c in  
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16 parallel with changes in TS, even though ferritin is not changed,<sup>32</sup> putting further  
17  
18 emphasis on the fact that NTBI/LPI reflected by TS is the proximal cause of the toxicity.  
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20 Several strategies are available to decrease iron, including chelation therapy and  
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22 phlebotomy. Phlebotomy is an easy, inexpensive, and well-tolerated intervention.  
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24 Reduction in TS by phlebotomy has been shown to improve measures of diabetic  
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26 control.<sup>32</sup> Furthermore, correction of severe iron overload can significantly improve  
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28 glucose tolerance.<sup>35</sup> Thus, the finding that a baseline measure of high TS as point  
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30 measure of toxic free iron plus elevation of ferritin, evidence of elevated cytosolic iron  
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32 over a longer period of time, predicts increased risk of mortality among individuals with  
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34 prediabetes supports the premise that toxic free iron is a health risk.  
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40 The normoglycemic group with elevated iron markers did not show increased  
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42 mortality risk in comparison to the reference group of normoglycemic and normal iron  
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44 marker levels. This may seem inconsistent with other data on the increased mortality risk  
45  
46 due to elevated TS by itself. However, there is the potential that the effect of TS on  
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48 mortality is modified by the presence of other variables.<sup>36,37</sup> This effect has been shown in  
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50 the past. Rather than being inconsistent with the TS alone and mortality findings, these  
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52 new findings enhance our understanding of elevated iron markers and morbidity and  
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3 mortality and allow us to consider the more complex, but real, situation of patients by  
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5 considering multiple variables together rather than independently.  
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8 This study has several limitations. First, although we have a nationally  
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10 representative, population-based cohort followed through the National Death Index, the  
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12 biomarkers are measured only at baseline. There is the possibility that either the  
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14 hyperglycemia or elevated iron measures were identified and interventions were  
15  
16 implemented to lower these biomarkers. If that were the case and a substantial number of  
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18 individuals did drop their levels due to interventions thereby decreasing the potential  
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20 mortality risk, the observed adjusted risk individuals elevated at baseline is even more  
21  
22 concerning. Second, we were only able to follow these individuals for 12 years. It is  
23  
24 possible that this time frame may have been too short to adequately see an effect for a  
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26 biomarker like prediabetes. However, we did censor the first three years of mortality so  
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28 that any deaths in that time frame would not be attributed to prediabetes. The model still  
29  
30 found a substantial mortality risk for the prediabetes plus iron markers in this length of  
31  
32 time. Third, we were unable to evaluate the relationship between being elevated on both  
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34 transferrin saturation and serum ferritin with prediabetes on the risk of mortality. We  
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36 attempted such an analysis but the number of individuals in the group with prediabetes  
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38 and elevation on both iron markers was small and the population estimates were deemed  
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40 unreliable.  
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48 In conclusion, this study representative of the population of the United States  
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50 helps to clarify the current evidence on the mortality risk of prediabetes and provides  
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52 further support for the role of elevated iron markers in health risk. Future screening and  
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3 intervention programs for prediabetes may benefit from additional strategies to recognize  
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5 and treat iron elevations, particularly transferrin saturation.  
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## CONTRIBUTION STATEMENT

AGM: conception, design, analysis, and drafting of manuscript

RJT: design, analysis, and drafting of manuscript

TDC: conception, design, and drafting of manuscript

RB: design and analysis

## COMPETING INTERESTS

The authors report no competing interests.

## DATA SHARING STATEMENT

Data from this study are publicly available on the Internet through the National Center for Health Statistics.

## FIGURE LEGENDS

Figure 1. Kaplan Meier curve of survival among individuals with prediabetes or normal glycemic levels.

— Normoglycemia

— Prediabetes

Figure 2. Kaplan Meier curve of survival among individuals with prediabetes and elevated transferrin saturation

- Normoglycemia and Normal Transferrin Saturation
- Normoglycemia and Elevated Transferrin Saturation
- Prediabetes and Normal Transferrin Saturation
- Prediabetes and Elevated Transferrin Saturation

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**REFERENCES**

1. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 2012;35:S64–S71.
2. Bullard KM, Saydah SH, Imperatore G, et al. Secular changes in U.S. Prediabetes prevalence defined by hemoglobin A1c and fasting plasma glucose: National Health and Nutrition Examination Surveys, 1999-2010. *Diabetes Care* 2013;36:2286–93.
3. Mainous AG 3rd, Tanner RJ, Baker R, et al. Prevalence of prediabetes in England from 2003 to 2011: population-based, cross-sectional study. *BMJ Open* 2014 Jun 9;4(6):e005002. doi: 10.1136/bmjopen-2014-005002. Accessed 29 June, 2014.
4. Centers for Disease Control and Prevention (CDC). Awareness of prediabetes--United States, 2005-2010. *MMWR Morb Mortal Wkly Rep*. 2013 Mar 22;62:209-12.
5. de Vegt F, Dekker JM, Ruhé HG, et al. Hyperglycaemia is associated with all-cause and cardiovascular mortality in the Hoorn population: the Hoorn Study. *Diabetologia* 1999;42:926–31.
6. Fuller JH, Shipley MJ, Rose G, et al. Coronary-heart-disease risk and impaired glucose tolerance. The Whitehall study. *Lancet* 1980;1:1373–76.
7. Valdés S, Botas P, Delgado E, Cadórniga FD. Mortality risk in Spanish adults with diagnosed diabetes, undiagnosed diabetes, or pre-diabetes. The asturias study 1998-2004. *Rev Esp Cardiol (Engl Ed)* 2009;62:528–34.
8. Deedwania P, Patel K, Fonarow GC, et al. Prediabetes is not an independent risk factor for incident heart failure, other cardiovascular events or mortality in older adults: findings from a population-based cohort study. *Int J Cardiol* 2013;168:3616–22.

- 1  
2  
3 9. Kowall B, Rathmann W, Heier M, et al. Categories of glucose tolerance and  
4 continuous glycemic measures and mortality. *Eur J Epidemiol* 2011;26:637–45.  
5  
6  
7  
8 10. Zhou XH, Qiao Q, Zethelius B, et al. Diabetes, prediabetes and cancer mortality.  
9  
10 *Diabetologia* 2010;53:1867–76.  
11  
12  
13 11. Barr EL, Zimmet PZ, Welborn TA, et al. Risk of cardiovascular and all-cause  
14 mortality in individuals with diabetes mellitus, impaired fasting glucose, and impaired  
15 glucose tolerance: the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab).  
16  
17 *Circulation* 2007;116:151–57.  
18  
19  
20  
21  
22 12. Orban E, Schwab S, Thorand B, Huth C. Association of iron indices and type 2  
23 diabetes: a meta-analysis of observational studies. *Diabetes Metab Res Rev* 2014;30:372–  
24  
25 94.  
26  
27  
28  
29 13. Bao W, Rong Y, Rong S, Liu L. Dietary iron intake, body iron stores, and the risk of  
30 type 2 diabetes: a systematic review and meta-analysis. *BMC Med* 2012;10:119. doi:  
31  
32 10.1186/1741-7015-10-119. Accessed 8 July, 2014.  
33  
34  
35  
36 14. Ellervik C, Mandrup-Poulsen T, Tybjærg-Hansen A, Nordestgaard BG. Total and  
37 cause-specific mortality by elevated transferrin saturation and hemochromatosis genotype  
38 in individuals with diabetes: two general population studies. *Diabetes Care* 2014;37:444–  
39  
40 52.  
41  
42  
43  
44  
45 15. Masuda Y, Ichii H, Vaziri ND. At pharmacologically relevant concentrations  
46 intravenous iron preparations cause pancreatic beta cell death. *Am J Transl Res*.  
47  
48 2013;6:64-70.  
49  
50  
51  
52  
53  
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57  
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51  
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55  
56  
57  
58  
59  
60
16. Ellervik C, Tybjaerg-Hansen A, Nordestgaard BG. Total mortality by transferrin saturation levels: Two general population studies and a meta analysis. *Clin Chem* 2011;57:459–466.
17. Kim KS, Son HG, Hong NS, Lee DH. Associations of serum ferritin and transferrin % saturation with all-cause, cancer, and cardiovascular disease mortality: Third National Health and Nutrition Examination Survey follow-up study. *J Prev Med Public Health* 2012;45:196-203.
18. Mainous AG 3rd, Gill JM, Carek PJ. Elevated transferrin saturation and mortality. *Ann Fam Med* 2004;2:133–138.
19. Mainous AG 3rd, Wells B, Carek PJ, et al. The mortality risk of elevated serum transferrin saturation and consumption of dietary iron. *Ann Fam Med* 2004;2:139-44.
20. Stack AG, Mutwali AI, Nguyen HT, et al. Transferrin saturation ratio and risk of total and cardiovascular mortality in the general population. *QJM*. 2014;107:623-33.
21. Wells BJ, Mainous AG 3rd, King DE, et al. The combined effect of transferrin saturation and low density lipoprotein on mortality. *Fam Med* 2004;36:324-9.
22. Ellervik C, Marott JL, Tybjærg-Hansen A, Schnohr P, Nordestgaard BG. Total and Cause-Specific Mortality by Moderately and Markedly Increased Ferritin Concentrations: General Population Study and Metaanalysis. *Clin Chem*. 2014 Aug 25. pii: clinchem.2014.229013.
23. Ponikowska B, Suchocki T, Paleczny B, et al. Iron status and survival in diabetic patients with coronary artery disease. *Diabetes Care* 2013;36:4147–56.

- 1  
2  
3 24. Morris DH, Khunti K, Achana F, et al. Progression rates from HbA(1c) 6.0-6.4% and  
4 other prediabetes definitions to type 2 diabetes: a meta-analysis. *Diabetologia*  
5  
6 2013;56:1489–93.  
7  
8  
9  
10 25. National Center for Health Statistics. Office of Analysis and Epidemiology, NCHS  
11  
12 2011 Linked Mortality Files Matching Methodology, September, 2013. Hyattsville,  
13  
14 Maryland.  
15  
16 [http://www.cdc.gov/nchs/data/datalinkage/2011\\_linked\\_mortality\\_file\\_matching\\_method](http://www.cdc.gov/nchs/data/datalinkage/2011_linked_mortality_file_matching_methodology.pdf)  
17  
18 [ology.pdf](http://www.cdc.gov/nchs/data/datalinkage/2011_linked_mortality_file_matching_methodology.pdf). Accessed 20 August, 2014.  
19  
20  
21  
22 26. Carson AP1, Fox CS, McGuire DK, Levitan EB, Laclaustra M, Mann DM, Muntner  
23  
24 P. Low hemoglobin A1c and risk of all-cause mortality among US adults without  
25  
26 diabetes. *Circ Cardiovasc Qual Outcomes*. 2010 Nov;3(6):661-7.  
27  
28  
29 27. Ellervik C, Andersen HU, Tybjærg-Hansen A, et al. Total mortality by elevated  
30  
31 transferrin saturation in patients with diabetes. *Diabetes Care* 2013;36:2646–54.  
32  
33  
34 28. Adams PC, Reboussin DM, Barton JC, et al. Hemochromatosis and Iron Overload  
35  
36 Screening (HEIRS) Study Research Investigators. Hemochromatosis and iron-overload  
37  
38 screening in a racially diverse population. *N Engl J Med*. 2005;352:1769-78.  
39  
40  
41 29. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and  
42  
43 cardiovascular disease: application to clinical and public health practice. A statement for  
44  
45 healthcare professionals from the Centers for Disease Control and Prevention and the  
46  
47 American Heart Association. *Circulation*. 2003;107(3):499 – 511.  
48  
49  
50 30. Mainous AG 3rd, Wright RU, Hulihan MM, et al. Elevated transferrin saturation,  
51  
52 health-related quality of life and telomere length. *Biometals* 2014;27:135–41  
53  
54  
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59  
60
31. Portero McLellan KC, Wyne K, Villagomez ET, Hsueh WA. Therapeutic interventions to reduce the risk of progression from prediabetes to type 2 diabetes mellitus. *Ther Clin Risk Manag* 2014;10:173–88.
32. Fernández-Real JM, Peñarroja G, Castro A, García-Bragado F, Hernández-Aguado I, Ricart W. Blood letting in high-ferritin type 2 diabetes: effects on insulin sensitivity and beta-cell function. *Diabetes* 2002;51:1000–4.
33. Hansen JB, Moen IW, Mandrup-Poulsen T. Iron: the hard player in diabetes pathophysiology. *Acta Physiol* 2014;210:717–32.
34. Coates TD. Physiology and pathophysiology of iron in hemoglobin-associated diseases. *Free Radic Biol Med* 2014;72C:23–40.
35. Farmaki K, Tzoumari I, Pappa C, et al. Normalisation of total body iron load with very intensive combined chelation reverses cardiac and endocrine complications of thalassaemia major. *Br J Haematol* 2010;148:466–75.
36. Mainous AG 3rd, Wells B, Carek PJ, Gill JM, Geesey ME. The mortality risk of elevated serum transferrin saturation and consumption of dietary iron. *Ann Fam Med*. 2004;2:139-44.
37. Wells BJ, Mainous AG 3rd, King DE, Gill JM, Carek PJ, Geesey ME. The combined effect of transferrin saturation and low density lipoprotein on mortality. *Fam Med*. 2004;36(5):324-9.

Table 1: Baseline Characteristics of the Sample

	<b>Study Sample (%)</b>
<b>Unweighted Sample Size</b>	8,003
<b>Population Estimate</b>	80,653,788
<b>Age, years</b>	
40-54	49.6
55-69	31.2
70+	19.2
<b>Sex, male</b>	46.1
<b>Race/Ethnicity</b>	
Non-Hispanic White	82.2
Non-Hispanic Black	8.3
Hispanic	6.7
Asian/Other	2.9
<b>Has health insurance</b>	93.6
<b>Obese</b>	23.3
<b>Current Smoker</b>	23.6
<b>Has diagnosed high cholesterol</b>	40.1
<b>Has diagnosed hypertension</b>	31.3
<b>Ever diagnosed with heart attack</b>	5.3
<b>Ever diagnosed with stroke</b>	2.8
<b>Ever diagnosed with cancer</b>	12.3
<b>Relative with diabetes</b>	41.2
<b>Relative with heart attack before age 50</b>	15.4
<b>Elevated C-reactive protein</b>	1.2
<b>Elevated Transferrin Saturation</b>	3.3
<b>Elevated Ferritin</b>	15.6
<b>Prediabetes</b>	23.2
<b>Assumed deceased</b>	27.0

Table 2: Adjusted Hazard Ratios from Cox Regression for Mortality Risk of Individuals with Prediabetes

	<b>Hazard Ratio</b>	<b>95% Confidence Interval</b>
<b>Adjusted Model<sup>a</sup></b>		
Prediabetes	1.04	1.00-1.08
Normoglycemia	1.0	--

<sup>a</sup>Adjusted for: age, gender, race/ethnicity, health insurance status, obesity, current smoking status, diagnosed heart attack, diagnosed stroke, diagnosed hypertension, diagnosed high cholesterol, diagnosis of cancer, family history of diabetes, and family history of early heart attack.

Table 3: Adjusted Hazard Ratios from Cox Regression for Mortality Risk of Individuals with Prediabetes and Iron Markers

	Hazard Ratio	95% Confidence Interval
<b>Adjusted Model<sup>a</sup></b>		
Normal Transferrin Saturation/ Normoglycemia	1.00	--
Normal Transferrin Saturation/ Prediabetes	1.13	1.09-1.18
Elevated Transferrin Saturation/ Normoglycemia	.99	.90-1.08
Elevated Transferrin Saturation/ Prediabetes	1.86	1.05-3.29
<b>Adjusted Model<sup>b</sup></b>		
Normal Ferritin/ Normoglycemia	1.00	--
Normal Ferritin/ Prediabetes	1.15	1.09-1.20
Elevated Ferritin/ Normoglycemia	1.05	1.00-1.12
Elevated Ferritin/ Prediabetes	1.14	1.04-1.24

<sup>a</sup>Adjusted for: age, gender, race/ethnicity, health insurance status, obesity, current smoking status, diagnosed heart attack, diagnosed stroke, diagnosed hypertension, diagnosed high cholesterol, diagnosis of cancer, family history of diabetes, and family history of early heart attack.

<sup>b</sup>Adjusted for: age, gender, race/ethnicity, health insurance status, obesity, current smoking status, diagnosed heart attack, diagnosed stroke, diagnosed hypertension, diagnosed high cholesterol, diagnosis of cancer, family history of diabetes, family history of early heart attack, and elevated C-reactive protein.

## Prediabetes, Elevated Iron, and All-Cause Mortality: A Cohort Study

Running Title: Prediabetes, Elevated Iron, and All-Cause Mortality

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## ABSTRACT

**Objectives:** Data have indicated low to nonexistent increased mortality risk for individuals with prediabetes, but it is unclear if the risk is increased when the patient has elevated iron markers. Our purpose was to examine the mortality risk among adults with prediabetes in the context of co-existing elevated transferrin saturation (TS) or serum ferritin.

**Setting:** Data collected by the third National Health and Nutrition Examination Survey 1988-1994 (NHANES III) in the United States and by the National Center for Health Statistics for the National Death Index from 1988-2006.

**Participants:** Individuals age 40 and older who participated in the NHANES and provided a blood sample.

Primary Outcome Variable: Mortality was measured as all-cause mortality.

**Results:** Adjusted analyses show that prediabetes has a small increased mortality risk (HR =1.04; 95% CI, 1.00-1.08). Persons who had prediabetes and elevated serum ferritin had an increased hazard ratio for death (HR=1.14; 95% CI, 1.04-1.24) compared with those who had normal ferritin and normal glucose. Among persons with prediabetes who had elevated TS, they had an increased mortality risk (HR=1.86; 95% CI, 1.05-3.29) compared with those with normal TS levels and normal glucose. **Conclusion:** The mortality risk of prediabetes is low. However, among individuals who have co-existing elevated iron markers, particularly transferrin saturation, the risk rises substantially.



## STRENGTHS AND LIMITATIONS OF STUDY

- This study utilizes a nationally representative population-based cohort that allows generalization of results to the population of the United States.
- Mortality was left-censored to control for the potential misclassification of people who were very ill at baseline.
- Biomarkers for elevated iron and prediabetes were available only at baseline.
- We were able to only observe individuals for 12 years, which may be insufficient to adequately see an effect on mortality for prediabetes.

## INTRODUCTION

Prediabetes is defined by blood glucose concentrations that are higher than normal, but lower than established thresholds for diabetes.<sup>1</sup> Prediabetes is a high risk state for the development of not only diabetes, but also associated complications. Recent data have shown that in developed countries such as the United States and the United Kingdom more than a third of adults have prediabetes but the vast majority of these do not realize it.<sup>2-4</sup>

Several studies have shown that prediabetes is a mortality risk.<sup>5,6</sup> On the other hand, other studies have found that prediabetes is not a mortality risk.<sup>7-10</sup> Still other studies showed that the relationship was not very robust and was dependent on the measure of prediabetes.<sup>11</sup>

Three different meta-analyses of observational studies have concluded that elevated iron indices like serum ferritin and transferrin saturation (TS) are strongly associated with increased risk for developing diabetes.<sup>12-14</sup> Some evidence exists to indicate that pancreatic beta cells are killed in the presence of iron.<sup>15</sup> In addition to increased risk of diabetes, elevated TS or elevated ferritin is associated with increased mortality in the general population.<sup>16-22</sup> Further, recent evidence suggests that among patients with diabetes, mortality risk increases in the presence of elevated ferritin or elevated TS.<sup>23,24</sup> We therefore hypothesize that the mortality risk of individuals with prediabetes will be increased in the presence of elevated iron markers.

Considering that only about 10% of the US population with prediabetes realize that they have prediabetes, a better understanding of the potential mortality risk is warranted. Consequently, the purpose of this study was to evaluate the association

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3 between prediabetes, elevated serum ferritin, elevated TS and mortality in a large,  
4 nationally representative cohort.  
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## 7 8 **METHODS**

9 We conducted longitudinal analyses of the third National Health and Nutrition  
10 Examination Survey, 1988-1994 (NHANES III) linked to mortality data collected  
11 through the National Death Index. Mortality data were available through December 31,  
12 2006. The NHANES III survey provides population estimates of the United States and  
13 was conducted from October, 1988 through October, 1994. The NHANES III used  
14 complex, multi-stage, stratified, clustered samples of civilian, noninstitutionalized  
15 population, Of the 39,695 individuals eligible to participate, a total of 30,818 persons  
16 were examined in their homes or in mobile examination centers (MEC) which visited 89  
17 communities across the United States (a participation rate of 77.6%). The health  
18 examination included collection of blood and urine specimens for the conduct of various  
19 laboratory analyses. The NHANES provides preexisting de-identified public use data  
20 which do not need specific approval from the National Center for Health Statistics.  
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37 The NHANES III data merged with the National Death Index is a prospective  
38 cohort study that passively followed up on the participants in the NHANES III. The  
39 linked mortality file uses a probabilistic matching method.<sup>25</sup> We limited our study to  
40 individuals 40 years old and older at baseline, the time of their NHANES III interview.  
41 The National Death Index involves searching national databases containing information  
42 about mortality and causes of death. Mortality status was ascertained by computerized  
43 matching to national databases and evaluation of the resulting matches. Persons not found  
44 to be deceased were assumed alive for analytic purposes. All living survey participants  
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3 had been observed for 146 months, and our survival analysis was carried out to  
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6 December 31, 2006.

### 7 8 *Previously Diagnosed Diabetes*

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10 The NHANES III assessed participants for diagnosed diabetes using the  
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12 questions, “Have you ever been told by a doctor that you have diabetes or sugar  
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14 diabetes?”, “Were you pregnant when you were told that you had diabetes?” and “Other  
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16 than during pregnancy, has a doctor ever told you that you have diabetes or sugar  
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18 diabetes?” We defined participants as having diagnosed diabetes if they answered “yes”  
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20 to ever having been told they had diabetes, excluding pregnancy. Individuals with  
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22 previously diagnosed diabetes were removed from the analysis. We also removed  
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24 individuals with an HbA1c of 6.5% or greater, to account for undiagnosed diabetes.  
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### 29 *Normoglycemia & Prediabetes*

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31 We defined normoglycemia as an HbA1c level between 4.0%--5.6% (20-38 mmol/mol).  
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33 To control for any potential effect of low HbA1c, we also removed individuals with an  
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35 HbA1c below 4.0% (20 mmol/mol), a level associated with increased all-cause mortality  
36  
37 in adults without diabetes.<sup>26</sup>  
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41 We defined prediabetes among individuals without previously diagnosed diabetes  
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43 using HbA1c ranges as specified by the American Diabetes Association, 5.7%–6.4% (39-  
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45 46 mmol/mol).<sup>1</sup> This range has been shown in a meta-analysis to be predictive of  
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47 progression to diabetes.<sup>19</sup> We excluded individuals with previously diagnosed diabetes  
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49 because the current glycemic status of those patients may simply represent diabetes  
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51 control. Prediabetes status was missing for 1,637 of the NHANES respondents over the  
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60 age of 40.

### *Transferrin Saturation*

Serum iron and total iron-binding capacity (TIBC) were measured in serum, and calculated by dividing serum iron by TIBC and multiplying by 100. For the analyses, elevated transferrin saturation was categorized as  $TS \geq 50\%$ . Individuals with TS below 25% were removed from the analysis, as low TS has been linked to increased risk of mortality.<sup>23</sup> Despite the lack of universal agreement on the upper and lower limits of normal TS, these cut points has been used in several studies evaluating diabetes, TS and mortality.<sup>23,27</sup> Data were missing for transferrin saturation level for 536 of the NHANES respondents over the age of 40.

### *Serum Ferritin*

Serum ferritin was used as a measure of body iron stores and was measured using the QuantImmune Ferritin IRMA kit. Serum ferritin was categorized for the analyses as elevated if it was  $\geq 674.1$  pmol/l (300 ng/mL) for males and  $\geq 449.4$  pmol/l (200 ng/mL) for females.<sup>28</sup> Individuals with serum ferritin below 56.175 pmol/l (25 ng/mL) were removed from the analysis, as low ferritin has been linked to an increased risk of mortality.<sup>23</sup> Data were missing for serum ferritin level for 539 of the NHANES respondents over the age of 40.

### *Mortality*

Mortality was measured as all-cause mortality. Mortality status was ascertained solely by computerized matching to national databases and evaluation of the resulting matches.

### *Covariates*

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Covariates used in our analyses included: age at baseline in the NHANES III, gender, race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, and other race), health insurance status, obesity (Body Mass Index computed in the exam of  $\geq 30$ ), previous diagnosis of a heart attack, previous diagnosis of a stroke, previous diagnosis of hypertension, previous diagnosis of hypercholesterolemia, previous diagnosis of cancer, family history of diabetes, family history of myocardial infarction before age 50, and current smoking status. Respondents were considered non-smokers if they reported smoking at least 100 cigarettes in their life or if they had smoked more than 100 cigarettes and were not currently smoking.

In the analysis of serum ferritin, we also controlled for C-reactive protein. Ferritin is an acute phase reactant as well as an indicator of iron stores and as such may indicate inflammation. Consequently, we controlled for inflammation by adjusting for C-reactive protein. C-reactive protein was considered elevated at levels above 3.0 mg/l.<sup>29</sup>

#### *Analysis*

In an effort to control for potential misclassification of persons who were very ill at baseline thereby affecting mortality risk of prediabetes, we left-censored the analysis to exclude any mortality events that occurred in the first three years following the individuals examination for the first three years of the cohort. 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. This strategy of basing the analyses on population estimates is a characteristic that makes the NHANES different from many other cohort designs that do not use weighted population estimates. However, this strategy does provide national

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3 **generalizability.** Because of the complex sampling design of the survey, we performed  
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5 statistical analyses using the statistical software package SUDAAN (Research Triangle  
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7 Institute, Raleigh, NC), as recommended by the National Center for Health Statistics  
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9 **(NCHS).** Using SAS (Cary, NC), we computed Kaplan-Meier plots to show graphically  
10  
11 the unadjusted relationship between all-cause mortality and prediabetes.  
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15 **All analyses were based on the population estimates and we followed the NCHS**  
16  
17 **recommendations for assessing the reliability of estimates in the context of a limited**  
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19 **sample size. If the standard error of an estimate was greater than 30% of an estimate it**  
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21 **would be considered unreliable All estimates met the criteria for reliability.**  
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25 To accomplish our goals of examining a possible synergistic effect of having  
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27 elevated iron with prediabetes we classified the population into 4 groups based upon  
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29 prediabetes or normoglycemia and normal and elevated TS. We also classified the  
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31 population into 4 groups based upon prediabetes or normoglycemia and normal and  
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33 elevated iron based on levels of TS and ferritin.  
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37 We performed Cox proportional hazards analyses to measure the associations  
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39 between all-cause mortality and prediabetes controlling for all of the studied covariates  
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41 using listwise deletion to account for missing data. In these models, survival time was a  
42  
43 continuous variable measured in 1-month increments from the date of the exam.  
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47 **We also performed adjusted Cox proportional hazards analysis with all-cause**  
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49 **mortality for prediabetes in the 4 part variables with TS adjusting for the aforementioned**  
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51 **covariates. For the adjusted Cox proportional hazards analysis with ferritin we adjusted**  
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53 **for the aforementioned covariates and also C-reactive protein.**  
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3 We evaluated the proportionality of the hazards through examination of the  
4 Schoenfeld residuals.  
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## 7 8 RESULTS 9

10 A total of 8,003 (unweighted) individuals were over 40 years old and had HbA1c  
11 levels between 4.0 and 6.4%. Baseline characteristics for the sample are shown in Table  
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13 1. Table 1 indicates that 23.2% of the weighted sample had prediabetes, 15.6% of the  
14 sample exhibited elevated serum ferritin, and 3.3% had elevated TS.  
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18 Of the respondents that had prediabetes, 38.8% died within 12 years, compared to  
19 23.4% of respondents with normal HbA1c levels. Among individuals with normal TS  
20 and normoglycemia, 23.1% died, compared to 23.7% of those with elevated TS, 37.5% of  
21 those with normal TS and prediabetes, and 44.7% of those with elevated TS and  
22 prediabetes. Among individuals with normal ferritin and normoglycemia, 34.3% died,  
23 compared with 38.8% of those with normal ferritin and prediabetes, 29.2% of those with  
24 elevated ferritin and normoglycemia, and 38.8% of those with elevated ferritin and  
25 prediabetes.  
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38 Table 2 shows the results the adjusted Cox proportional hazards model for  
39 prediabetes. Table 2 indicates prediabetes alone has a small increased mortality risk. The  
40 Kaplan Meier curve of the survival and prediabetes over the length of the time under  
41 observation is shown in Fig 1.  
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48 Table 3 presents results of the analyses combining prediabetes with iron markers.  
49 In models that examined the impact of a prediabetes state combined with markers of low  
50 iron, the hazard ratios were similar to that of prediabetes alone. However, when  
51 combined with prediabetes, there was an increased mortality risk among individuals with  
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3 TS  $\geq 50$ , as well as with individuals who had increased ferritin. The risk was most  
4 increased when individuals had elevated ferritin and elevated transferrin saturation  
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6 together. Figure 2 represents the relationship of survival of the four groups over the 12  
7  
8 years under observation. Individuals with prediabetes in the presence of elevated iron  
9  
10 have lower survival probabilities than other groups. An examination of the Schoenfeld  
11  
12 residuals suggested proportionality of hazards and appropriateness of the statistical model  
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14 for these analyses.  
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## 20 21 DISCUSSION

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23 The results of this study in a nationally representative cohort that followed  
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25 individuals for 12 years confirm that the mortality risk of prediabetes is probably low.  
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27 This is not unexpected based on the mixed results from previous studies, several of which  
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29 found either no future mortality risk or risk that was not robust across measures.  
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31 However, we found that the presence of transferrin saturation and serum ferritin is  
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33 associated with increased mortality risk of individuals with prediabetes. Among  
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35 individuals with normal iron levels, those with prediabetes had low mortality risk levels  
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37 similar to the adjusted risk of prediabetes alone. On the other hand, in adjusted survival  
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39 analyses, individuals with prediabetes who also had elevated transferrin saturation had  
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41 substantially increased mortality risk. These findings extend previous work on iron  
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43 markers and diabetes to the previously uninvestigated area of prediabetes.  
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49 These results suggest that additional stratification of individuals with prediabetes  
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51 on the basis of iron markers would be useful to identify those with higher risk and who  
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53 might benefit from iron lowering therapies. Previous data has indicated that elevated iron  
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55 markers are associated with the development of diabetes and that among individuals with  
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3 diabetes the co-occurrence of elevated TS increases those patients' mortality risk. Early  
4 identification of individuals with both conditions (prediabetes, elevated iron) may help in  
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6 both slowing the development of diabetes as well as decreasing mortality risk. It is  
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8 important for early identification of these individuals because much like individuals with  
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10 prediabetes, the vast majority of individuals with elevated iron do not know it.<sup>30</sup> These  
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12 individuals need to be identified to mitigate the increased risk posed by elevated iron in  
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14 combination with prediabetes. Such individuals would be targets for intensive  
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16 interventions to reduce risk, including typical lifestyle interventions shown to help avoid  
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18 the onset of diabetes in people at high risk.<sup>31</sup> Although more research is needed into the  
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20 ability of interventions on iron in prediabetes to affect development of diabetes and  
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22 mortality risk, some data suggest that reduction of TS improves HbA1c and glucose  
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24 control.<sup>32</sup>

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32 These associations of TS and ferritin with mortality in the context of prediabetes  
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34 are not surprising especially if elevations of these parameters are interpreted in light of  
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36 current understanding of iron toxicity.<sup>33</sup> Iron, whether absorbed as iron salts or in dietary  
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38 heme is processed by enterocytes and released into the plasma where it is transported in a  
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40 non-reactive state bound to transferrin. Iron that is bound to transferrin is in the  $\text{Fe}^{+3}$   
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42 state and is not reactive and, therefore, not toxic. However, when TS is above 40 to 50%,  
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44 free iron or so-called non-transferrin-bound iron (NTBI) is released into the plasma as the  
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46 buffering ability of transferrin is exceeded.<sup>34</sup> Labile plasma iron (LPI) is a highly  
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48 reactive subspecies of NTBI that interacts with hydrogen peroxide through Fenton  
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50 chemistry to form the extremely powerful oxidants, hydroxyl radical and singlet oxygen.  
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52 These are the free radicals that ultimately directly damage protein and DNA. Perhaps  
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3 more importantly, NTBI/LPI species are able to enter cells via ion channels. These  
4 channels, unlike the transferrin receptor, are not regulated so this reactive iron freely  
5 enters the cytoplasm of the pancreas, pituitary, and heart.  
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10 The current results suggest that exposure to excessive free iron is dangerous in the  
11 context of prediabetes. Furthermore, elevated ferritin and TS predict poor diabetes  
12 control and phlebotomy to reduce iron even over short periods of time improve HbA1c in  
13 parallel with changes in TS, even though ferritin is not changed,<sup>32</sup> putting further  
14 emphasis on the fact that NTBI/LPI reflected by TS is the proximal cause of the toxicity.  
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22 Several strategies are available to decrease iron, including chelation therapy and  
23 phlebotomy. Phlebotomy is an easy, inexpensive, and well-tolerated intervention.  
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27 Reduction in TS by phlebotomy has been shown to improve measures of diabetic  
28 control.<sup>32</sup> Furthermore, correction of severe iron overload can significantly improve  
29 glucose tolerance.<sup>35</sup> Thus, the finding that a baseline measure of high TS as point  
30 measure of toxic free iron plus elevation of ferritin, evidence of elevated cytosolic iron  
31 over a longer period of time, predicts increased risk of mortality among individuals with  
32 prediabetes supports the premise that toxic free iron is a health risk.  
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41 The normoglycemic group with elevated iron markers did not show increased  
42 mortality risk in comparison to the reference group of normoglycemic and normal iron  
43 marker levels. This may seem inconsistent with other data on the increased mortality risk  
44 due to elevated TS by itself. However, there is the potential that the effect of TS on  
45 mortality is modified by the presence of other variables.<sup>36,37</sup> This effect has been shown in  
46 the past. Rather than being inconsistent with the TS alone and mortality findings, these  
47 new findings enhance our understanding of elevated iron markers and morbidity and  
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3 mortality and allow us to consider the more complex, but real, situation of patients by  
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5 considering multiple variables together rather than independently.  
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8 This study has several limitations. First, although we have a nationally  
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10 representative, population-based cohort followed through the National Death Index, the  
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12 biomarkers are measured only at baseline. There is the possibility that either the  
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14 hyperglycemia or elevated iron measures were identified and interventions were  
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16 implemented to lower these biomarkers. If that were the case and a substantial number of  
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18 individuals did drop their levels due to interventions thereby decreasing the potential  
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20 mortality risk, the observed adjusted risk individuals elevated at baseline is even more  
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22 concerning. Second, we were only able to follow these individuals for 12 years. It is  
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24 possible that this time frame may have been too short to adequately see an effect for a  
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26 biomarker like prediabetes. However, we did censor the first three years of mortality so  
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28 that any deaths in that time frame would not be attributed to prediabetes. The model still  
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30 found a substantial mortality risk for the prediabetes plus iron markers in this length of  
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32 time. Third, we were unable to evaluate the relationship between being elevated on both  
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34 transferrin saturation and serum ferritin with prediabetes on the risk of mortality. We  
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36 attempted such an analysis but the number of individuals in the group with prediabetes  
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38 and elevation on both iron markers was small and the population estimates were deemed  
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40 unreliable.  
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48 In conclusion, this study representative of the population of the United States  
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50 helps to clarify the current evidence on the mortality risk of prediabetes and provides  
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52 further support for the role of elevated iron markers in health risk. Future screening and  
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3 intervention programs for prediabetes may benefit from additional strategies to recognize  
4 and treat iron elevations, particularly transferrin saturation.  
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## CONTRIBUTION STATEMENT

AGM: conception, design, analysis, and drafting of manuscript

RJT: design, analysis, and drafting of manuscript

TDC: conception, design, and drafting of manuscript

RB: design and analysis

## COMPETING INTERESTS

The authors report no competing interests.

**REFERENCES**

1. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 2012;35:S64–S71.
2. Bullard KM, Saydah SH, Imperatore G, et al. Secular changes in U.S. Prediabetes prevalence defined by hemoglobin A1c and fasting plasma glucose: National Health and Nutrition Examination Surveys, 1999-2010. *Diabetes Care* 2013;36:2286–93.
3. Mainous AG 3rd, Tanner RJ, Baker R, et al. Prevalence of prediabetes in England from 2003 to 2011: population-based, cross-sectional study. *BMJ Open* 2014 Jun 9;4(6):e005002. doi: 10.1136/bmjopen-2014-005002. Accessed 29 June, 2014.
4. Centers for Disease Control and Prevention (CDC). Awareness of prediabetes--United States, 2005-2010. *MMWR Morb Mortal Wkly Rep*. 2013 Mar 22;62:209-12.
5. de Vegt F, Dekker JM, Ruhé HG, et al. Hyperglycaemia is associated with all-cause and cardiovascular mortality in the Hoorn population: the Hoorn Study. *Diabetologia* 1999;42:926–31.
6. Fuller JH, Shipley MJ, Rose G, et al. Coronary-heart-disease risk and impaired glucose tolerance. The Whitehall study. *Lancet* 1980;1:1373–76.
7. Valdés S, Botas P, Delgado E, Cadórniga FD. Mortality risk in Spanish adults with diagnosed diabetes, undiagnosed diabetes, or pre-diabetes. The asturias study 1998-2004. *Rev Esp Cardiol (Engl Ed)* 2009;62:528–34.
8. Deedwania P, Patel K, Fonarow GC, et al. Prediabetes is not an independent risk factor for incident heart failure, other cardiovascular events or mortality in older adults: findings from a population-based cohort study. *Int J Cardiol* 2013;168:3616–22.

- 1  
2  
3 9. Kowall B, Rathmann W, Heier M, et al. Categories of glucose tolerance and  
4 continuous glycemic measures and mortality. *Eur J Epidemiol* 2011;26:637–45.  
5  
6  
7  
8 10. Zhou XH, Qiao Q, Zethelius B, et al. Diabetes, prediabetes and cancer mortality.  
9  
10 *Diabetologia* 2010;53:1867–76.  
11  
12  
13 11. Barr EL, Zimmet PZ, Welborn TA, et al. Risk of cardiovascular and all-cause  
14 mortality in individuals with diabetes mellitus, impaired fasting glucose, and impaired  
15 glucose tolerance: the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab).  
16  
17 *Circulation* 2007;116:151–57.  
18  
19  
20  
21  
22 12. Orban E, Schwab S, Thorand B, Huth C. Association of iron indices and type 2  
23 diabetes: a meta-analysis of observational studies. *Diabetes Metab Res Rev* 2014;30:372–  
24  
25 94.  
26  
27  
28  
29 13. Bao W, Rong Y, Rong S, Liu L. Dietary iron intake, body iron stores, and the risk of  
30 type 2 diabetes: a systematic review and meta-analysis. *BMC Med* 2012;10:119. doi:  
31  
32 10.1186/1741-7015-10-119. Accessed 8 July, 2014.  
33  
34  
35  
36 14. Ellervik C, Mandrup-Poulsen T, Tybjærg-Hansen A, Nordestgaard BG. Total and  
37 cause-specific mortality by elevated transferrin saturation and hemochromatosis genotype  
38 in individuals with diabetes: two general population studies. *Diabetes Care* 2014;37:444–  
39  
40 52.  
41  
42  
43  
44  
45 15. Masuda Y, Ichii H, Vaziri ND. At pharmacologically relevant concentrations  
46 intravenous iron preparations cause pancreatic beta cell death. *Am J Transl Res*.  
47  
48 2013;6:64-70.  
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60
16. Ellervik C, Tybjaerg-Hansen A, Nordestgaard BG. Total mortality by transferrin saturation levels: Two general population studies and a meta analysis. *Clin Chem* 2011;57:459–466.
17. Kim KS, Son HG, Hong NS, Lee DH. Associations of serum ferritin and transferrin % saturation with all-cause, cancer, and cardiovascular disease mortality: Third National Health and Nutrition Examination Survey follow-up study. *J Prev Med Public Health* 2012;45:196-203.
18. Mainous AG 3rd, Gill JM, Carek PJ. Elevated transferrin saturation and mortality. *Ann Fam Med* 2004;2:133–138.
19. Mainous AG 3rd, Wells B, Carek PJ, et al. The mortality risk of elevated serum transferrin saturation and consumption of dietary iron. *Ann Fam Med* 2004;2:139-44.
20. Stack AG, Mutwali AI, Nguyen HT, et al. Transferrin saturation ratio and risk of total and cardiovascular mortality in the general population. *QJM*. 2014;107:623-33.
21. Wells BJ, Mainous AG 3rd, King DE, et al. The combined effect of transferrin saturation and low density lipoprotein on mortality. *Fam Med* 2004;36:324-9.
22. Ellervik C, Marott JL, Tybjærg-Hansen A, Schnohr P, Nordestgaard BG. Total and Cause-Specific Mortality by Moderately and Markedly Increased Ferritin Concentrations: General Population Study and Metaanalysis. *Clin Chem*. 2014 Aug 25. pii: clinchem.2014.229013.
23. Ponikowska B, Suchocki T, Paleczny B, et al. Iron status and survival in diabetic patients with coronary artery disease. *Diabetes Care* 2013;36:4147–56.

1  
2  
3 24. Morris DH, Khunti K, Achana F, et al. Progression rates from HbA(1c) 6.0-6.4% and  
4 other prediabetes definitions to type 2 diabetes: a meta-analysis. *Diabetologia*  
5  
6 2013;56:1489–93.  
7

8  
9  
10 25. National Center for Health Statistics. Office of Analysis and Epidemiology, NCHS  
11 2011 Linked Mortality Files Matching Methodology, September, 2013. Hyattsville,  
12 Maryland.  
13 [http://www.cdc.gov/nchs/data/datalinkage/2011\\_linked\\_mortality\\_file\\_matching\\_method](http://www.cdc.gov/nchs/data/datalinkage/2011_linked_mortality_file_matching_methodology.pdf)  
14 [ology.pdf](http://www.cdc.gov/nchs/data/datalinkage/2011_linked_mortality_file_matching_methodology.pdf). Accessed 20 August, 2014.  
15  
16  
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21

22 26. Carson AP1, Fox CS, McGuire DK, Levitan EB, Laclaustra M, Mann DM, Muntner  
23 P. Low hemoglobin A1c and risk of all-cause mortality among US adults without  
24 diabetes. *Circ Cardiovasc Qual Outcomes*. 2010 Nov;3(6):661-7.  
25  
26  
27

28 27. Ellervik C, Andersen HU, Tybjærg-Hansen A, et al. Total mortality by elevated  
29 transferrin saturation in patients with diabetes. *Diabetes Care* 2013;36:2646–54.  
30  
31  
32

33 28. Adams PC, Reboussin DM, Barton JC, et al. Hemochromatosis and Iron Overload  
34 Screening (HEIRS) Study Research Investigators. Hemochromatosis and iron-overload  
35 screening in a racially diverse population. *N Engl J Med*. 2005;352:1769-78.  
36  
37  
38

39 29. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and  
40 cardiovascular disease: application to clinical and public health practice. A statement for  
41 healthcare professionals from the Centers for Disease Control and Prevention and the  
42 American Heart Association. *Circulation*. 2003;107(3):499 – 511.  
43  
44  
45  
46  
47  
48

49 30. Mainous AG 3rd, Wright RU, Hulihan MM, et al. Elevated transferrin saturation,  
50 health-related quality of life and telomere length. *Biometals* 2014;27:135–41  
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31. Portero McLellan KC, Wyne K, Villagomez ET, Hsueh WA. Therapeutic interventions to reduce the risk of progression from prediabetes to type 2 diabetes mellitus. *Ther Clin Risk Manag* 2014;10:173–88.
32. Fernández-Real JM, Peñarroja G, Castro A, García-Bragado F, Hernández-Aguado I, Ricart W. Blood letting in high-ferritin type 2 diabetes: effects on insulin sensitivity and beta-cell function. *Diabetes* 2002;51:1000–4.
33. Hansen JB, Moen IW, Mandrup-Poulsen T. Iron: the hard player in diabetes pathophysiology. *Acta Physiol* 2014;210:717–32.
34. Coates TD. Physiology and pathophysiology of iron in hemoglobin-associated diseases. *Free Radic Biol Med* 2014;72C:23–40.
35. Farmaki K, Tzoumari I, Pappa C, et al. Normalisation of total body iron load with very intensive combined chelation reverses cardiac and endocrine complications of thalassaemia major. *Br J Haematol* 2010;148:466–75.
36. Mainous AG 3rd, Wells B, Carek PJ, Gill JM, Geesey ME. The mortality risk of elevated serum transferrin saturation and consumption of dietary iron. *Ann Fam Med*. 2004;2:139-44.
37. Wells BJ, Mainous AG 3rd, King DE, Gill JM, Carek PJ, Geesey ME. The combined effect of transferrin saturation and low density lipoprotein on mortality. *Fam Med*. 2004;36(5):324-9.

Table 1: Baseline Characteristics of the Sample

	<b>Study Sample (%)</b>
<b>Unweighted Sample Size</b>	<b>8,003</b>
<b>Population Estimate</b>	<b>80,653,788</b>
<b>Age, years</b>	
40-54	49.6
55-69	31.2
70+	19.2
<b>Sex, male</b>	<b>46.1</b>
<b>Race/Ethnicity</b>	
Non-Hispanic White	82.2
Non-Hispanic Black	8.3
Hispanic	6.7
Asian/Other	2.9
<b>Has health insurance</b>	<b>93.6</b>
<b>Obese</b>	<b>23.3</b>
<b>Current Smoker</b>	<b>23.6</b>
<b>Has diagnosed high cholesterol</b>	<b>40.1</b>
<b>Has diagnosed hypertension</b>	<b>31.3</b>
<b>Ever diagnosed with heart attack</b>	<b>5.3</b>
<b>Ever diagnosed with stroke</b>	<b>2.8</b>
<b>Ever diagnosed with cancer</b>	<b>12.3</b>
<b>Relative with diabetes</b>	<b>41.2</b>
<b>Relative with heart attack before age 50</b>	<b>15.4</b>
<b>Elevated C-reactive protein</b>	<b>1.2</b>
<b>Elevated Transferrin Saturation</b>	<b>3.3</b>
<b>Elevated Ferritin</b>	<b>15.6</b>
<b>Prediabetes</b>	<b>23.2</b>
<b>Assumed deceased</b>	<b>27.0</b>

Table 2: Adjusted Hazard Ratios from Cox Regression for Mortality Risk of Individuals with Prediabetes

	<b>Hazard Ratio</b>	<b>95% Confidence Interval</b>
<b>Adjusted Model<sup>a</sup></b>		
Prediabetes	1.04	1.00-1.08
Normoglycemia	1.0	--

<sup>a</sup>Adjusted for: age, gender, race/ethnicity, health insurance status, obesity, current smoking status, diagnosed heart attack, diagnosed stroke, diagnosed hypertension, diagnosed high cholesterol, diagnosis of cancer, family history of diabetes, and family history of early heart attack.

Table 3: Adjusted Hazard Ratios from Cox Regression for Mortality Risk of Individuals with Prediabetes and Iron Markers

	Hazard Ratio	95% Confidence Interval
<b>Adjusted Model<sup>a</sup></b>		
Normal Transferrin Saturation/ Normoglycemia	1.00	--
Normal Transferrin Saturation/ Prediabetes	1.13	1.09-1.18
Elevated Transferrin Saturation/ Normoglycemia	.99	.90-1.08
Elevated Transferrin Saturation/ Prediabetes	1.86	1.05-3.29
<b>Adjusted Model<sup>b</sup></b>		
Normal Ferritin/ Normoglycemia	1.00	--
Normal Ferritin/ Prediabetes	1.15	1.09-1.20
Elevated Ferritin/ Normoglycemia	1.05	1.00-1.12
Elevated Ferritin/ Prediabetes	1.14	1.04-1.24

<sup>a</sup>Adjusted for: age, gender, race/ethnicity, health insurance status, obesity, current smoking status, diagnosed heart attack, diagnosed stroke, diagnosed hypertension, diagnosed high cholesterol, diagnosis of cancer, family history of diabetes, and family history of early heart attack.

<sup>b</sup>Adjusted for: age, gender, race/ethnicity, health insurance status, obesity, current smoking status, diagnosed heart attack, diagnosed stroke, diagnosed hypertension, diagnosed high cholesterol, diagnosis of cancer, family history of diabetes, family history of early heart attack, and elevated C-reactive protein.

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3 Figure 1. Kaplan Meier curve of survival among individuals with prediabetes or normal  
4 glyceimic levels.  
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8 ——— Normoglycemia

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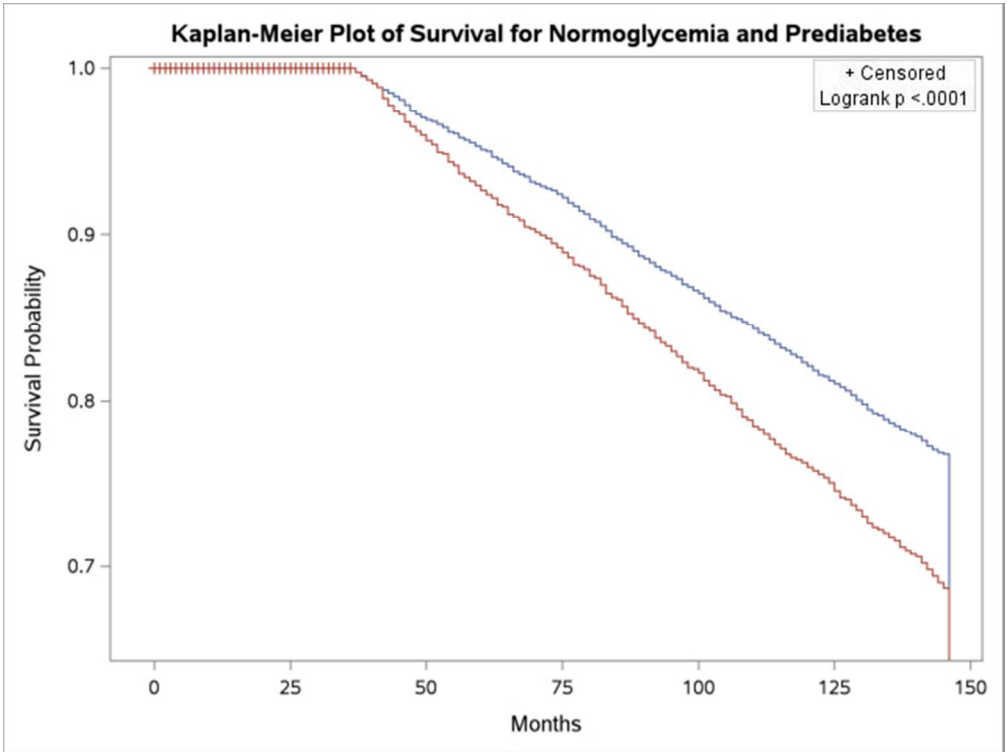


Figure 1. Kaplan Meier curve of survival among individuals with prediabetes or normal glyceic levels.  
blue line = Normoglycemia  
red line = Prediabetes

54x40mm (300 x 300 DPI)

For peer review only



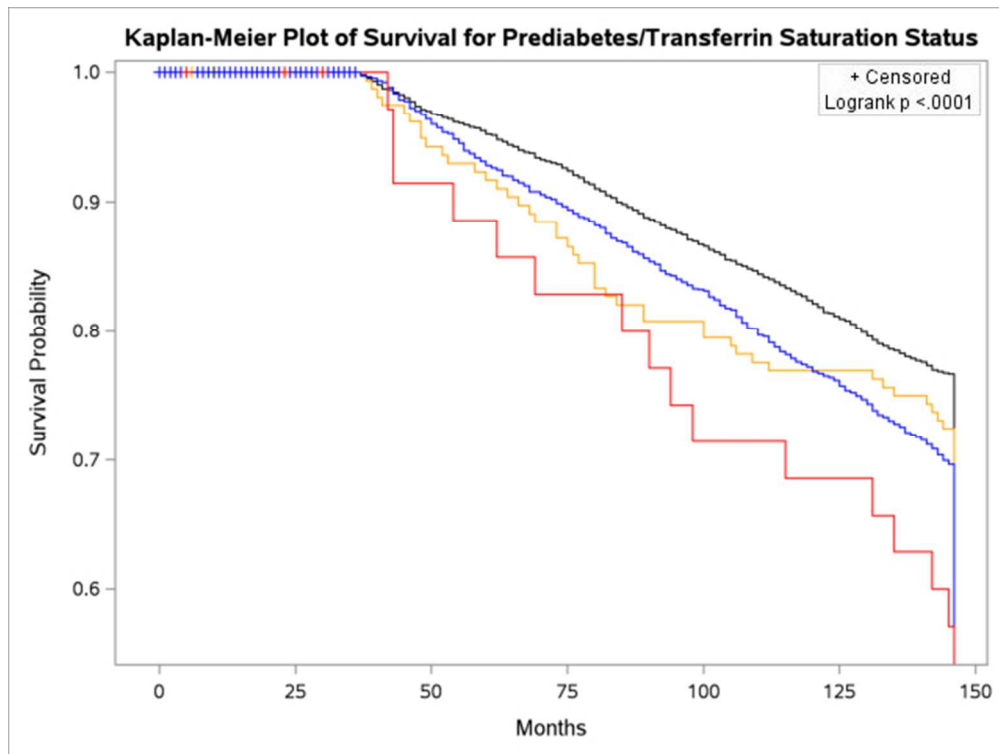


Figure 2. Kaplan Meier curve of survival among individuals with prediabetes and elevated transferrin saturation

black line = Normoglycemia and Normal Transferrin Saturation  
 yellow line = Normoglycemia and Elevated Transferrin Saturation  
 blue line = Prediabetes and Normal Transferrin Saturation  
 red line = Prediabetes and Elevated Transferrin Saturation

54x40mm (300 x 300 DPI)

only

## STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page number
<b>Title and abstract</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
<b>Introduction</b>			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4-5
<b>Methods</b>			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	5
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-8
Bias	9	Describe any efforts to address potential sources of bias	9
Study size	10	Explain how the study size was arrived at	5-8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	8-9
		(b) Describe any methods used to examine subgroups and interactions	8
		(c) Explain how missing data were addressed	8
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	NA
		(e) Describe any sensitivity analyses	NA

Continued on next page

<b>Results</b>			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5, 9
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9, 19
		(b) Indicate number of participants with missing data for each variable of interest	6-7
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	5
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	19
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-10 20- 22
		(b) Report category boundaries when continuous variables were categorized	NA
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	10- 11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12- 13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).

# BMJ Open

## Prediabetes, Elevated Iron, and All-Cause Mortality: A Cohort Study

Journal:	<i>BMJ Open</i>
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<b>Primary Subject Heading</b>:	Diabetes and endocrinology
Secondary Subject Heading:	Haematology (incl blood transfusion)
Keywords:	General diabetes < DIABETES & ENDOCRINOLOGY, HAEMATOLOGY, DIABETES & ENDOCRINOLOGY

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Manuscripts

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3 **Prediabetes, Elevated Iron, and All-Cause Mortality: A Cohort Study**  
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5 Running Title: Prediabetes, Elevated Iron, and All-Cause Mortality  
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52 Main Text Word Count: 3114

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54 Abstract Word Count: 211  
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56  
57 **Key Words:** prediabetes, transferrin saturation, ferritin, NHANES, mortality  
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## ABSTRACT

**Objectives:** Data have indicated low to nonexistent increased mortality risk for individuals with prediabetes, but it is unclear if the risk is increased when the patient has elevated iron markers. Our purpose was to examine the mortality risk among adults with prediabetes in the context of co-existing elevated transferrin saturation (TS) or serum ferritin.

**Setting:** Data collected by the third National Health and Nutrition Examination Survey 1988-1994 (NHANES III) in the United States and by the National Center for Health Statistics for the National Death Index from 1988-2006.

**Participants:** Individuals age 40 and older who participated in the NHANES and provided a blood sample.

**Primary Outcome Variable:** Mortality was measured as all-cause mortality.

**Results:** Adjusted analyses show that prediabetes has a small increased mortality risk (HR =1.04; 95% CI, 1.00-1.08). Persons who had prediabetes and elevated serum ferritin had an increased hazard ratio for death (HR=1.14; 95% CI, 1.04-1.24) compared with those who had normal ferritin and normal glucose. Among persons with prediabetes who had elevated TS, they had an increased mortality risk (HR=1.88; 95% CI, 1.06-3.30) compared with those with normal TS levels and normal glucose.

**Conclusion:** The mortality risk of prediabetes is low. However, among individuals who have co-existing elevated iron markers, particularly transferrin saturation, the risk rises substantially.

## STRENGTHS AND LIMITATIONS OF STUDY

- This study utilizes a nationally representative population-based cohort that allows generalization of results to the noninstitutionalized civilian population of the United States.
- Mortality was left-censored to control for the potential misclassification of people who were very ill at baseline.
- Biomarkers for elevated iron and prediabetes were available only at baseline.
- We were able to only observe individuals for 12 years, which may be insufficient to adequately see an effect on mortality for prediabetes.

## INTRODUCTION

Prediabetes is defined by blood glucose concentrations that are higher than normal, but lower than established thresholds for diabetes.<sup>1</sup> Prediabetes is a high risk state for the development of not only diabetes, but also associated complications. Recent data have shown that in developed countries such as the United States and the United Kingdom more than a third of adults have prediabetes but the vast majority of these do not realize it.<sup>2-4</sup>

Several studies have shown that prediabetes is a mortality risk.<sup>5,6</sup> On the other hand, other studies have found that prediabetes is not a mortality risk.<sup>7-10</sup> Still other studies showed that the relationship was not very robust and was dependent on the measure of prediabetes.<sup>11</sup>

Three different meta-analyses of observational studies have concluded that elevated iron indices like serum ferritin and transferrin saturation (TS) are strongly associated with increased risk for developing diabetes.<sup>12-14</sup> Some evidence exists to indicate that pancreatic beta cells are killed in the presence of iron.<sup>15</sup> In addition to increased risk of diabetes, elevated TS or elevated ferritin is associated with increased mortality in the general population.<sup>16-22</sup> Further, recent evidence suggests that among patients with diabetes, mortality risk increases in the presence of elevated ferritin or elevated TS.<sup>23,24</sup> We therefore hypothesize that the mortality risk of individuals with prediabetes will be increased in the presence of elevated iron markers.

Considering that only about 10% of the US population with prediabetes realize that they have prediabetes, a better understanding of the potential mortality risk is warranted. Consequently, the purpose of this study was to evaluate the association



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3 between prediabetes, elevated serum ferritin, elevated TS and mortality in a large,  
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5 nationally representative cohort.  
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## 8 **METHODS**

9 We conducted longitudinal analyses of the third National Health and Nutrition  
10 Examination Survey, 1988-1994 (NHANES III) linked to mortality data collected  
11 through the National Death Index. Mortality data were available through December 31,  
12 2006. The NHANES III survey provides population estimates of the United States and  
13 was conducted from October, 1988 through October, 1994.  
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21 The NHANES III used complex, multi-stage, stratified, clustered samples of  
22 civilian, noninstitutionalized population and is designed and conducted for the purpose of  
23 making health-related prevalence estimates that are nationally generalizable. To make  
24 accurate population estimates, analysis of the NHANES requires the use of weight and  
25 design variables that account for this complex design. The use of sampling weights is  
26 necessary to account for differences in probability of selection for each participant and  
27 also accounts for noncoverage and nonresponse.<sup>25</sup> The NHANES III oversampled  
28 different groups, including older individuals, African Americans, and Mexican-  
29 Americans. The application of sampling weights allows us to conduct analyses on the  
30 individuals who were sampled in the NHANES and extrapolate those results to the  
31 population at large. According to the technical reports provided by the National Center  
32 for Health Statistics, without the use of sampling weights, misinterpretation of population  
33 estimates based on NHANES III is likely. This strategy of basing the analyses on  
34 population estimates is a characteristic that makes the NHANES different from many  
35 other cohort designs that do not use weighted population estimates, and provides national  
36 generalizability.  
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Of the 39,695 individuals eligible to participate, a total of 30,818 persons of all ages were examined in their homes or in mobile examination centers (MEC) which visited 89 communities across the United States (a participation rate of 77.6%). The health examination included collection of blood and urine specimens for the conduct of various laboratory analyses.

The NHANES III data merged with the National Death Index is a prospective cohort study that passively followed up on the participants in the NHANES III. The linked mortality file uses a probabilistic matching method.<sup>26</sup> The National Death Index involves searching national databases containing information about mortality and causes of death. Mortality status was ascertained by computerized matching to national databases and evaluation of the resulting matches. Persons not found to be deceased were assumed alive for analytic purposes.

The NHANES III is preexisting de-identified public use data which do not need specific approval from the National Center for Health Statistics.

We limited our study to individuals 40 years old and older at baseline, the time of their NHANES III interview. All analyses were based on the population estimates generated by applying variables accounting for the design and sampling methodology of the NHANES. The results presented here are generalizable to the noninstitutionalized civilian population of the United States aged 40 and older from 1998-1994.

#### *Previously Diagnosed Diabetes*

The NHANES III assessed participants for diagnosed diabetes using the questions, “Have you ever been told by a doctor that you have diabetes or sugar diabetes?”, “Were you pregnant when you were told that you had diabetes?” and “Other

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3 than during pregnancy, has a doctor ever told you that you have diabetes or sugar  
4 diabetes?" We defined participants as having diagnosed diabetes if they answered "yes"  
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6 to ever having been told they had diabetes, excluding pregnancy. Individuals with  
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8 previously diagnosed diabetes were removed from the analysis. We also removed  
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10 individuals with an HbA1c of 6.5% or greater, to account for undiagnosed diabetes.  
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#### 14 *Normoglycemia & Prediabetes*

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16 We defined normoglycemia as an HbA1c level between 4.0%--5.6% (20-38 mmol/mol).  
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18 To control for any potential effect of low HbA1c, we also removed individuals with an  
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20 HbA1c below 4.0% (20 mmol/mol), a level associated with increased all-cause mortality  
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22 in adults without diabetes.<sup>27</sup>  
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27 We defined prediabetes among individuals without previously diagnosed diabetes  
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29 using HbA1c ranges as specified by the American Diabetes Association, 5.7%–6.4% (39-  
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31 46 mmol/mol).<sup>1</sup> This range has been shown in a meta-analysis to be predictive of  
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33 progression to diabetes.<sup>19</sup> We excluded individuals with previously diagnosed diabetes  
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35 because the current glycemic status of those patients may simply represent diabetes  
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37 control. Prediabetes status was missing for 1,637 of the NHANES respondents over the  
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39 age of 40.  
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#### 43 *Transferrin Saturation*

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45 Serum iron and total iron-binding capacity (TIBC) were measured in serum, and  
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47 calculated by dividing serum iron by TIBC and multiplying by 100. For the analyses,  
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49 elevated transferrin saturation was categorized as TS  $\geq$ 50%. Individuals with TS below  
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51 25% were removed from the analysis, as low TS has been linked to increased risk of  
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53 mortality.<sup>23</sup> Despite the lack of universal agreement on the upper and lower limits of  
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3 normal TS, these cut points has been used in several studies evaluating diabetes, TS and  
4 mortality.<sup>23,28</sup> Data were missing for transferrin saturation level for 536 of the NHANES  
5 respondents over the age of 40.  
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### 10 *Serum Ferritin*

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12 Serum ferritin was used as a measure of body iron stores and was measured using  
13 the QuantImmune Ferritin IRMA kit. Serum ferritin was categorized for the analyses as  
14 elevated if it was  $\geq 674.1$  pmol/l (300 ng/mL) for males and  $\geq 449.4$  pmol/l (200 ng/mL)  
15 for females.<sup>29</sup> Individuals with serum ferritin below 56.175 pmol/l (25 ng/mL) were  
16 removed from the analysis, as low ferritin has been linked to an increased risk of  
17 mortality.<sup>23</sup> Data were missing for serum ferritin level for 539 of the NHANES  
18 respondents over the age of 40.  
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### 29 *Mortality*

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31 Mortality was measured as all-cause mortality. Mortality status was ascertained  
32 solely by computerized matching to national databases and evaluation of the resulting  
33 matches. All living survey participants examined in this study had been observed for 146  
34 months, and our survival analysis was carried out to December 31, 2006.  
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### 41 *Covariates*

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43 Covariates used in our analyses included: age at baseline in the NHANES III,  
44 gender, race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican-American, and  
45 other), health insurance status, obesity (Body Mass Index computed in the exam of  $\geq 30$ ),  
46 previous diagnosis of a heart attack, previous diagnosis of a stroke, previous diagnosis of  
47 hypertension, previous diagnosis of hypercholesterolemia, previous diagnosis of cancer,  
48 family history of diabetes, family history of myocardial infarction before age 50, and  
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3 current smoking status. Respondents were considered non-smokers if they reported  
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5 smoking less than 100 cigarettes in their life or if they had smoked more than 100  
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7 cigarettes and were not currently smoking.  
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10 In the analysis of serum ferritin, we also controlled for C-reactive protein. Ferritin  
11 is an acute phase reactant as well as an indicator of iron stores and as such may indicate  
12 inflammation. Consequently, we controlled for inflammation by adjusting for C-reactive  
13 protein. C-reactive protein was considered elevated at levels above 3.0 mg/l.<sup>30</sup>  
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### 19 *Analysis*

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21 In an effort to control for potential misclassification of persons who were very ill  
22 at baseline thereby affecting mortality risk of prediabetes, we left-censored the analysis to  
23 exclude any mortality events that occurred in the first three years following the  
24 individuals examination for the first three years of the cohort. Because of the complex  
25 sampling design of the survey, we performed statistical analyses using the statistical  
26 software package SUDAAN (Research Triangle Institute, Raleigh, NC), as recommended  
27 by the National Center for Health Statistics (NCHS). Using SAS (Cary, NC), we  
28 computed Kaplan-Meier plots to show graphically the unadjusted relationship between  
29 all-cause mortality and prediabetes.  
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43 We followed the NCHS recommendations for assessing the reliability of estimates  
44 in the context of a limited sample size. If the standard error of an estimate was greater  
45 than 30% of an estimate it would be considered unreliable All estimates met the criteria  
46 for reliability.  
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52 To accomplish our goals of examining a possible synergistic effect of having  
53 elevated iron with prediabetes we classified the population into 4 groups based upon  
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3 prediabetes or normoglycemia and normal or elevated TS. The population was also  
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5 classified into 4 groups based upon prediabetes or normoglycemia and normal or elevated  
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7 serum ferritin.  
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10 We performed Cox proportional hazards analyses to measure the associations  
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12 between all-cause mortality and prediabetes controlling for all of the studied covariates  
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14 using listwise deletion to account for missing data. In these models, survival time was a  
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16 continuous variable measured in 1-month increments from the date of the exam.  
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19 We also performed adjusted Cox proportional hazards analysis with all-cause  
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21 mortality for prediabetes in the 4 part variables with TS adjusting for the aforementioned  
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23 covariates. For the adjusted Cox proportional hazards analysis with ferritin we adjusted  
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25 for the aforementioned covariates and also C-reactive protein.  
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28 We evaluated the proportionality of the hazards through examination of the  
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30 Schoenfeld residuals.  
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## 33 **RESULTS**

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35 A total of 8,003 (unweighted) individuals were over 40 years old and had HbA1c  
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37 levels between 4.0 and 6.4%, or 80,653,788 individuals nationally. Baseline  
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39 characteristics for the sample are shown in Table 1. Table 1 indicates that 23.2% of the  
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41 weighted sample had prediabetes, 15.6% of the sample exhibited elevated serum ferritin,  
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43 and 3.3% had elevated TS.  
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47 Of the respondents that had prediabetes, 38.8% died within 12 years (723,702  
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49 died; 11,431,597 survived), compared to 23.4% of respondents with normal HbA1c  
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51 levels (14,527,028 died; 47,458,061 survived). Among individuals with normal TS and  
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53 normoglycemia, 23.1% died (10,724,279 died; 35,649,283 survived), compared to 23.7%  
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3 of those with elevated TS and normoglycemia (412,237 died; 1,327,253 survived), 37.5%  
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5 of those with normal TS and prediabetes (5,137,131 died; 8,572,762 survived), and  
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7 44.7% of those with elevated TS and prediabetes (126,633 died; 156,790 survived).  
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9 Among individuals with normal ferritin and normoglycemia, 24.3% died (10,967,486  
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11 died; 34,132,718 survived), compared with 38.8% of those with normal ferritin and  
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13 prediabetes (5,465,483 died; 8,614,683 survived), 29.2% of those with elevated ferritin  
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15 and normoglycemia (2,333,436 died; 5,662,576 survived), and 38.8% of those with  
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17 elevated ferritin and prediabetes (1,150,647 died; 1,818,565 survived).  
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22 Table 2 shows the results the adjusted Cox proportional hazards model for  
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24 prediabetes. Table 2 indicates prediabetes alone has a small increased mortality risk. The  
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26 Kaplan Meier curve of the survival and prediabetes over the length of the time under  
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28 observation is shown in Fig 1.  
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32 Table 3 presents results of the analyses combining prediabetes with iron markers.  
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34 In models that examined the impact of a prediabetes state combined with markers of low  
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36 iron, the hazard ratios were similar to that of prediabetes alone. However, when  
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38 combined with prediabetes, there was an increased mortality risk among individuals with  
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40 TS  $\geq$ 50, as well as with individuals who had increased ferritin. The risk was most  
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42 increased when individuals had elevated ferritin and elevated transferrin saturation  
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44 together. Figure 2 represents the relationship of survival of the four groups over the 12  
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46 years under observation. Individuals with prediabetes in the presence of elevated iron  
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48 have lower survival probabilities than other groups. An examination of the Schoenfeld  
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50 residuals suggested proportionality of hazards and appropriateness of the statistical model  
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52 for these analyses.  
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## DISCUSSION

The results of this study in a nationally representative cohort that followed individuals for 12 years confirm that the mortality risk of prediabetes is probably low. This is not unexpected based on the mixed results from previous studies, several of which found either no future mortality risk or risk that was not robust across measures. However, we found that the presence of transferrin saturation and serum ferritin is associated with increased mortality risk of individuals with prediabetes. Among individuals with normal iron levels, those with prediabetes had low mortality risk levels similar to the adjusted risk of prediabetes alone. On the other hand, in adjusted survival analyses, individuals with prediabetes who also had elevated transferrin saturation had substantially increased mortality risk. These findings extend previous work on iron markers and diabetes to the previously uninvestigated area of prediabetes.

These results suggest that additional stratification of individuals with prediabetes on the basis of iron markers would be useful to identify those with higher risk and who might benefit from iron lowering therapies. Previous data has indicated that elevated iron markers are associated with the development of diabetes and that among individuals with diabetes the co-occurrence of elevated TS increases those patients' mortality risk. Early identification of individuals with both conditions (prediabetes, elevated iron) may help in both slowing the development of diabetes as well as decreasing mortality risk. It is important for early identification of these individuals because much like individuals with prediabetes, the vast majority of individuals with elevated iron do not know it.<sup>31</sup> These individuals need to be identified to mitigate the increased risk posed by elevated iron in combination with prediabetes. Such individuals would be targets for intensive



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3 interventions to reduce risk, including typical lifestyle interventions shown to help avoid  
4 the onset of diabetes in people at high risk.<sup>32</sup> Although more research is needed into the  
5 ability of interventions on iron in prediabetes to affect development of diabetes and  
6 mortality risk, some data suggest that reduction of TS improves HbA1c and glucose  
7 control.<sup>33</sup>

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These associations of TS and ferritin with mortality in the context of prediabetes are not surprising especially if elevations of these parameters are interpreted in light of current understanding of iron toxicity.<sup>34</sup> Iron, whether absorbed as iron salts or in dietary heme is processed by enterocytes and released into the plasma where it is transported in a non-reactive state bound to transferrin. Iron that is bound to transferrin is in the Fe<sup>+3</sup> state and is not reactive and, therefore, not toxic. However, when TS is above 40 to 50%, free iron or so-called non-transferrin-bound iron (NTBI) is released into the plasma as the buffering ability of transferrin is exceeded.<sup>35</sup> Labile plasma iron (LPI) is a highly reactive subspecies of NTBI that interacts with hydrogen peroxide through Fenton chemistry to form the extremely powerful oxidants, hydroxyl radical and singlet oxygen. These are the free radicals that ultimately directly damage protein and DNA. Perhaps more importantly, NTBI/LPI species are able to enter cells via ion channels. These channels, unlike the transferrin receptor, are not regulated so this reactive iron freely enters the cytoplasm of the pancreas, pituitary, and heart.

The current results suggest that exposure to excessive free iron is dangerous in the context of prediabetes. Furthermore, elevated ferritin and TS predict poor diabetes control and phlebotomy to reduce iron even over short periods of time improve HbA1c in parallel with changes in TS, even though ferritin is not changed,<sup>33</sup> putting further

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3 emphasis on the fact that NTBI/LPI reflected by TS is the proximal cause of the toxicity.  
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5 Several strategies are available to decrease iron, including chelation therapy and  
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7 phlebotomy. Phlebotomy is an easy, inexpensive, and well-tolerated intervention.  
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10 Reduction in TS by phlebotomy has been shown to improve measures of diabetic  
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12 control.<sup>33</sup> Furthermore, correction of severe iron overload can significantly improve  
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14 glucose tolerance.<sup>36</sup> Thus, the finding that a baseline measure of high TS as point  
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16 measure of toxic free iron plus elevation of ferritin, evidence of elevated cytosolic iron  
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18 over a longer period of time, predicts increased risk of mortality among individuals with  
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20 prediabetes supports the premise that toxic free iron is a health risk.  
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25 The normoglycemic group with elevated iron markers did not show increased  
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27 mortality risk in comparison to the reference group of normoglycemic and normal iron  
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29 marker levels. This may seem inconsistent with other data on the increased mortality risk  
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31 due to elevated TS by itself. However, there is the potential that the effect of TS on  
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33 mortality is modified by the presence of other variables.<sup>37,38</sup> This effect has been shown in  
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35 the past. Rather than being inconsistent with the TS alone and mortality findings, these  
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37 new findings enhance our understanding of elevated iron markers and morbidity and  
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39 mortality and allow us to consider the more complex, but real, situation of patients by  
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41 considering multiple variables together rather than independently.  
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46 This study has several limitations. First, although we have a nationally  
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48 representative, population-based cohort followed through the National Death Index, the  
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50 biomarkers are measured only at baseline. There is the possibility that either the  
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52 hyperglycemia or elevated iron measures were identified and interventions were  
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54 implemented to lower these biomarkers. If that were the case and a substantial number of  
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3 individuals did drop their levels due to interventions thereby decreasing the potential  
4 mortality risk, the observed adjusted risk individuals elevated at baseline is even more  
5 concerning. Second, we were only able to follow these individuals for 12 years. It is  
6 possible that this time frame may have been too short to adequately see an effect for a  
7 biomarker like prediabetes. However, we did censor the first three years of mortality so  
8 that any deaths in that time frame would not be attributed to prediabetes. The model still  
9 found a substantial mortality risk for the prediabetes plus iron markers in this length of  
10 time. Third, we were unable to evaluate the relationship between being elevated on both  
11 transferrin saturation and serum ferritin with prediabetes on the risk of mortality. We  
12 attempted such an analysis but the number of individuals in the group with prediabetes  
13 and elevation on both iron markers was small and the population estimates were deemed  
14 unreliable.

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32 In conclusion, this study representative of the population of the United States  
33 helps to clarify the current evidence on the mortality risk of prediabetes and provides  
34 further support for the role of elevated iron markers in health risk. Future screening and  
35 intervention programs for prediabetes may benefit from additional strategies to recognize  
36 and treat iron elevations, particularly transferrin saturation.  
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## CONTRIBUTION STATEMENT

AGM: conception, design, analysis, and drafting of manuscript

RJT: design, analysis, and drafting of manuscript

TDC: conception, design, and drafting of manuscript

RB: design and analysis

## COMPETING INTERESTS

The authors report no competing interests.

## DATA SHARING STATEMENT

Data from this study are publicly available on the Internet through the National Center for Health Statistics.

**REFERENCES**

1. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 2012;35:S64–S71.
2. Bullard KM, Saydah SH, Imperatore G, et al. Secular changes in U.S. Prediabetes prevalence defined by hemoglobin A1c and fasting plasma glucose: National Health and Nutrition Examination Surveys, 1999-2010. *Diabetes Care* 2013;36:2286–93.
3. Mainous AG 3rd, Tanner RJ, Baker R, et al. Prevalence of prediabetes in England from 2003 to 2011: population-based, cross-sectional study. *BMJ Open* 2014 Jun 9;4(6):e005002. doi: 10.1136/bmjopen-2014-005002. Accessed 29 June, 2014.
4. Centers for Disease Control and Prevention (CDC). Awareness of prediabetes--United States, 2005-2010. *MMWR Morb Mortal Wkly Rep*. 2013 Mar 22;62:209-12.
5. de Vegt F, Dekker JM, Ruhé HG, et al. Hyperglycaemia is associated with all-cause and cardiovascular mortality in the Hoorn population: the Hoorn Study. *Diabetologia* 1999;42:926–31.
6. Fuller JH, Shipley MJ, Rose G, et al. Coronary-heart-disease risk and impaired glucose tolerance. The Whitehall study. *Lancet* 1980;1:1373–76.
7. Valdés S, Botas P, Delgado E, Cadórniga FD. Mortality risk in Spanish adults with diagnosed diabetes, undiagnosed diabetes, or pre-diabetes. The asturias study 1998-2004. *Rev Esp Cardiol (Engl Ed)* 2009;62:528–34.
8. Deedwania P, Patel K, Fonarow GC, et al. Prediabetes is not an independent risk factor for incident heart failure, other cardiovascular events or mortality in older adults: findings from a population-based cohort study. *Int J Cardiol* 2013;168:3616–22.

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9. Kowall B, Rathmann W, Heier M, et al. Categories of glucose tolerance and continuous glycemic measures and mortality. *Eur J Epidemiol* 2011;26:637–45.
10. Zhou XH, Qiao Q, Zethelius B, et al. Diabetes, prediabetes and cancer mortality. *Diabetologia* 2010;53:1867–76.
11. Barr EL, Zimmet PZ, Welborn TA, et al. Risk of cardiovascular and all-cause mortality in individuals with diabetes mellitus, impaired fasting glucose, and impaired glucose tolerance: the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab). *Circulation* 2007;116:151–57.
12. Orban E, Schwab S, Thorand B, Huth C. Association of iron indices and type 2 diabetes: a meta-analysis of observational studies. *Diabetes Metab Res Rev* 2014;30:372–94.
13. Bao W, Rong Y, Rong S, Liu L. Dietary iron intake, body iron stores, and the risk of type 2 diabetes: a systematic review and meta-analysis. *BMC Med* 2012;10:119. doi: 10.1186/1741-7015-10-119. Accessed 8 July, 2014.
14. Ellervik C, Mandrup-Poulsen T, Tybjærg-Hansen A, Nordestgaard BG. Total and cause-specific mortality by elevated transferrin saturation and hemochromatosis genotype in individuals with diabetes: two general population studies. *Diabetes Care* 2014;37:444–52.
15. Masuda Y, Ichii H, Vaziri ND. At pharmacologically relevant concentrations intravenous iron preparations cause pancreatic beta cell death. *Am J Transl Res*. 2013;6:64-70.

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3  
4 16. Ellervik C, Tybjaerg-Hansen A, Nordestgaard BG. Total mortality by transferrin  
5 saturation levels: Two general population studies and a meta analysis. *Clin Chem*  
6 2011;57:459–466.  
7  
8  
9  
10 17. Kim KS, Son HG, Hong NS, Lee DH. Associations of serum ferritin and transferrin  
11 % saturation with all-cause, cancer, and cardiovascular disease mortality: Third National  
12 Health and Nutrition Examination Survey follow-up study. *J Prev Med Public Health*  
13 2012;45:196-203.  
14  
15  
16  
17 18. Mainous AG 3rd, Gill JM, Carek PJ. Elevated transferrin saturation and mortality.  
18 *Ann Fam Med* 2004;2:133–138.  
19  
20  
21 19. Mainous AG 3rd, Wells B, Carek PJ, et al. The mortality risk of elevated serum  
22 transferrin saturation and consumption of dietary iron. *Ann Fam Med* 2004;2:139-44.  
23  
24  
25 20. Stack AG, Mutwali AI, Nguyen HT, et al. Transferrin saturation ratio and risk of total  
26 and cardiovascular mortality in the general population. *QJM*. 2014;107:623-33.  
27  
28  
29 21. Wells BJ, Mainous AG 3rd, King DE, et al. The combined effect of transferrin  
30 saturation and low density lipoprotein on mortality. *Fam Med* 2004;36:324-9.  
31  
32  
33 22. Ellervik C, Marott JL, Tybjærg-Hansen A, Schnohr P, Nordestgaard BG. Total and  
34 Cause-Specific Mortality by Moderately and Markedly Increased Ferritin Concentrations:  
35 General Population Study and Metaanalysis. *Clin Chem*. 2014 Aug 25. pii:  
36 clinchem.2014.229013.  
37  
38  
39 23. Ponikowska B, Suchocki T, Paleczny B, et al. Iron status and survival in diabetic  
40 patients with coronary artery disease. *Diabetes Care* 2013;36:4147–56.  
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24. Morris DH, Khunti K, Achana F, et al. Progression rates from HbA(1c) 6.0-6.4% and other prediabetes definitions to type 2 diabetes: a meta-analysis. *Diabetologia* 2013;56:1489–93.
25. National Center for Health Statistics. Plan and operation of the Third National Health and Nutrition Examination Survey, 1988-1994. *Vital Health Stat.* 1994;1(32).  
[http://www.cdc.gov/nchs/data/series/sr\\_01/sr01\\_032.pdf](http://www.cdc.gov/nchs/data/series/sr_01/sr01_032.pdf). Accessed 15 October 2014.
26. National Center for Health Statistics. Office of Analysis and Epidemiology, NCHS 2011 Linked Mortality Files Matching Methodology, September, 2013. Hyattsville, Maryland.  
[http://www.cdc.gov/nchs/data/datalinkage/2011\\_linked\\_mortality\\_file\\_matching\\_methodology.pdf](http://www.cdc.gov/nchs/data/datalinkage/2011_linked_mortality_file_matching_methodology.pdf). Accessed 20 August, 2014.
27. Carson AP1, Fox CS, McGuire DK, Levitan EB, Laclaustra M, Mann DM, Muntner P. Low hemoglobin A1c and risk of all-cause mortality among US adults without diabetes. *Circ Cardiovasc Qual Outcomes.* 2010 Nov;3(6):661-7.
28. Ellervik C, Andersen HU, Tybjærg-Hansen A, et al. Total mortality by elevated transferrin saturation in patients with diabetes. *Diabetes Care* 2013;36:2646–54.
29. Adams PC, Reboussin DM, Barton JC, et al. Hemochromatosis and Iron Overload Screening (HEIRS) Study Research Investigators. Hemochromatosis and iron-overload screening in a racially diverse population. *N Engl J Med.* 2005;352:1769-78.
30. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice. A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation.* 2003;107(3):499 – 511.



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2  
3  
4 31. Mainous AG 3rd, Wright RU, Hulihan MM, et al. Elevated transferrin saturation,  
5 health-related quality of life and telomere length. *Biometals* 2014;27:135–41  
6  
7  
8 32. Portero McLellan KC, Wyne K, Villagomez ET, Hsueh WA. Therapeutic  
9 interventions to reduce the risk of progression from prediabetes to type 2 diabetes  
10 mellitus. *Ther Clin Risk Manag* 2014;10:173–88.  
11  
12  
13 33. Fernández-Real JM, Peñarroja G, Castro A, García-Bragado F, Hernández-Aguado I,  
14 Ricart W. Blood letting in high-ferritin type 2 diabetes: effects on insulin sensitivity and  
15 beta-cell function. *Diabetes* 2002;51:1000–4.  
16  
17  
18 34. Hansen JB, Moen IW, Mandrup-Poulsen T. Iron: the hard player in diabetes  
19 pathophysiology. *Acta Physiol* 2014;210:717–32.  
20  
21  
22 35. Coates TD. Physiology and pathophysiology of iron in hemoglobin-associated  
23 diseases. *Free Radic Biol Med* 2014;72C:23–40.  
24  
25  
26 36. Farmaki K, Tzoumari I, Pappa C, et al. Normalisation of total body iron load with  
27 very intensive combined chelation reverses cardiac and endocrine complications of  
28 thalassaemia major. *Br J Haematol* 2010;148:466–75.  
29  
30  
31 37. Mainous AG 3rd, Wells B, Carek PJ, Gill JM, Geesey ME. The mortality risk of  
32 elevated serum transferrin saturation and consumption of dietary iron. *Ann Fam Med*.  
33 2004;2:139-44.  
34  
35  
36 38. Wells BJ, Mainous AG 3rd, King DE, Gill JM, Carek PJ, Geesey ME. The combined  
37 effect of transferrin saturation and low density lipoprotein on mortality. *Fam Med*.  
38 2004;36(5):324-9.  
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Figure 1. Kaplan Meier curve of survival among individuals with prediabetes or normal glycaemic levels.

——— Normoglycemia

——— Prediabetes

Figure 2. Kaplan Meier curve of survival among individuals with prediabetes and elevated transferrin saturation

——— Normoglycemia and Normal Transferrin Saturation

——— Normoglycemia and Elevated Transferrin Saturation

——— Prediabetes and Normal Transferrin Saturation

——— Prediabetes and Elevated Transferrin Saturation

Table 1: Baseline Characteristics of the Sample

	<b>Study Sample (%)</b>
<b>Unweighted Sample Size</b>	8,003
<b>Population Estimate</b>	80,653,788
<b>Age, years</b>	
40-54	49.6
55-69	31.2
70+	19.2
<b>Sex, male</b>	46.1
<b>Race/Ethnicity</b>	
Non-Hispanic White	82.2
Non-Hispanic Black	8.3
Mexican-American	3.2
Other	6.4
<b>Has health insurance</b>	93.6
<b>Obese</b>	23.3
<b>Current Smoker</b>	23.6
<b>Has diagnosed high cholesterol</b>	40.1
<b>Has diagnosed hypertension</b>	31.3
<b>Ever diagnosed with heart attack</b>	5.3
<b>Ever diagnosed with stroke</b>	2.8
<b>Ever diagnosed with cancer</b>	12.3
<b>Relative with diabetes</b>	41.2
<b>Relative with heart attack before age 50</b>	15.4
<b>Elevated C-reactive protein</b>	1.2
<b>Elevated Transferrin Saturation</b>	3.3
<b>Elevated Ferritin</b>	15.6
<b>Prediabetes</b>	23.2
<b>Assumed deceased</b>	27.0

Table 2: Adjusted Hazard Ratios from Cox Regression for Mortality Risk of Individuals with Prediabetes

	<b>Hazard Ratio</b>	<b>95% Confidence Interval</b>
<b>Adjusted Model<sup>a</sup></b>		
Prediabetes	1.04	1.00-1.08
Normoglycemia	1.0	--

<sup>a</sup>Adjusted for: age, gender, race/ethnicity, health insurance status, obesity, current smoking status, diagnosed heart attack, diagnosed stroke, diagnosed hypertension, diagnosed high cholesterol, diagnosis of cancer, family history of diabetes, and family history of early heart attack.

Table 3: Adjusted Hazard Ratios from Cox Regression for Mortality Risk of Individuals with Prediabetes and Iron Markers

	Hazard Ratio	95% Confidence Interval
<b>Adjusted Model<sup>a</sup></b>		
Normal Transferrin Saturation/ Normoglycemia	1.00	--
Normal Transferrin Saturation/ Prediabetes	1.13	1.08-1.19
Elevated Transferrin Saturation/ Normoglycemia	.98	.90-1.07
Elevated Transferrin Saturation/ Prediabetes	1.88	1.06-3.30
<b>Adjusted Model<sup>b</sup></b>		
Normal Ferritin/ Normoglycemia	1.00	--
Normal Ferritin/ Prediabetes	1.15	1.09-1.20
Elevated Ferritin/ Normoglycemia	1.05	1.00-1.12
Elevated Ferritin/ Prediabetes	1.14	1.04-1.24

<sup>a</sup>Adjusted for: age, gender, race/ethnicity, health insurance status, obesity, current smoking status, diagnosed heart attack, diagnosed stroke, diagnosed hypertension, diagnosed high cholesterol, diagnosis of cancer, family history of diabetes, and family history of early heart attack.

<sup>b</sup>Adjusted for: age, gender, race/ethnicity, health insurance status, obesity, current smoking status, diagnosed heart attack, diagnosed stroke, diagnosed hypertension, diagnosed high cholesterol, diagnosis of cancer, family history of diabetes, family history of early heart attack, and elevated C-reactive protein.

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3 **Prediabetes, Elevated Iron, and All-Cause Mortality: A Cohort Study**  
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5 Running Title: Prediabetes, Elevated Iron, and All-Cause Mortality  
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## ABSTRACT

**Objectives:** Data have indicated low to nonexistent increased mortality risk for individuals with prediabetes, but it is unclear if the risk is increased when the patient has elevated iron markers. Our purpose was to examine the mortality risk among adults with prediabetes in the context of co-existing elevated transferrin saturation (TS) or serum ferritin.

**Setting:** Data collected by the third National Health and Nutrition Examination Survey 1988-1994 (NHANES III) in the United States and by the National Center for Health Statistics for the National Death Index from 1988-2006.

**Participants:** Individuals age 40 and older who participated in the NHANES and provided a blood sample.

**Primary Outcome Variable:** Mortality was measured as all-cause mortality.

**Results:** Adjusted analyses show that prediabetes has a small increased mortality risk (HR =1.04; 95% CI, 1.00-1.08). Persons who had prediabetes and elevated serum ferritin had an increased hazard ratio for death (HR=1.14; 95% CI, 1.04-1.24) compared with those who had normal ferritin and normal glucose. Among persons with prediabetes who had elevated TS, they had an increased mortality risk (HR=1.88; 95% CI, 1.06-3.30) compared with those with normal TS levels and normal glucose.

**Conclusion:** The mortality risk of prediabetes is low. However, among individuals who have co-existing elevated iron markers, particularly transferrin saturation, the risk rises substantially.



## STRENGTHS AND LIMITATIONS OF STUDY

- This study utilizes a nationally representative population-based cohort that allows generalization of results to the noninstitutionalized civilian population of the United States.
- Mortality was left-censored to control for the potential misclassification of people who were very ill at baseline.
- Biomarkers for elevated iron and prediabetes were available only at baseline.
- We were able to only observe individuals for 12 years, which may be insufficient to adequately see an effect on mortality for prediabetes.

## INTRODUCTION

Prediabetes is defined by blood glucose concentrations that are higher than normal, but lower than established thresholds for diabetes.<sup>1</sup> Prediabetes is a high risk state for the development of not only diabetes, but also associated complications. Recent data have shown that in developed countries such as the United States and the United Kingdom more than a third of adults have prediabetes but the vast majority of these do not realize it.<sup>2-4</sup>

Several studies have shown that prediabetes is a mortality risk.<sup>5,6</sup> On the other hand, other studies have found that prediabetes is not a mortality risk.<sup>7-10</sup> Still other studies showed that the relationship was not very robust and was dependent on the measure of prediabetes.<sup>11</sup>

Three different meta-analyses of observational studies have concluded that elevated iron indices like serum ferritin and transferrin saturation (TS) are strongly associated with increased risk for developing diabetes.<sup>12-14</sup> Some evidence exists to indicate that pancreatic beta cells are killed in the presence of iron.<sup>15</sup> In addition to increased risk of diabetes, elevated TS or elevated ferritin is associated with increased mortality in the general population.<sup>16-22</sup> Further, recent evidence suggests that among patients with diabetes, mortality risk increases in the presence of elevated ferritin or elevated TS.<sup>23,24</sup> We therefore hypothesize that the mortality risk of individuals with prediabetes will be increased in the presence of elevated iron markers.

Considering that only about 10% of the US population with prediabetes realize that they have prediabetes, a better understanding of the potential mortality risk is warranted. Consequently, the purpose of this study was to evaluate the association

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3 between prediabetes, elevated serum ferritin, elevated TS and mortality in a large,  
4 nationally representative cohort.  
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## 7 8 **METHODS**

9 We conducted longitudinal analyses of the third National Health and Nutrition  
10 Examination Survey, 1988-1994 (NHANES III) linked to mortality data collected  
11 through the National Death Index. Mortality data were available through December 31,  
12 2006. The NHANES III survey provides population estimates of the United States and  
13 was conducted from October, 1988 through October, 1994.  
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21 The NHANES III used complex, multi-stage, stratified, clustered samples of  
22 civilian, noninstitutionalized population and is designed and conducted for the purpose of  
23 making health-related prevalence estimates that are nationally generalizable. To make  
24 accurate population estimates, analysis of the NHANES requires the use of weight and  
25 design variables that account for this complex design. The use of sampling weights is  
26 necessary to account for differences in probability of selection for each participant and  
27 also accounts for noncoverage and nonresponse.<sup>25</sup> The NHANES III oversampled  
28 different groups, including older individuals, African Americans, and Mexican-  
29 Americans. The application of sampling weights allows us to conduct analyses on the  
30 individuals who were sampled in the NHANES and extrapolate those results to the  
31 population at large. According to the technical reports provided by the National Center  
32 for Health Statistics, without the use of sampling weights, misinterpretation of population  
33 estimates based on NHANES III is likely. This strategy of basing the analyses on  
34 population estimates is a characteristic that makes the NHANES different from many  
35 other cohort designs that do not use weighted population estimates, and provides national  
36 generalizability.  
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3 Of the 39,695 individuals eligible to participate, a total of 30,818 persons of all  
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Of the 39,695 individuals eligible to participate, a total of 30,818 persons of all ages were examined in their homes or in mobile examination centers (MEC) which visited 89 communities across the United States (a participation rate of 77.6%). The health examination included collection of blood and urine specimens for the conduct of various laboratory analyses.

The NHANES III data merged with the National Death Index is a prospective cohort study that passively followed up on the participants in the NHANES III. The linked mortality file uses a probabilistic matching method.<sup>26</sup> The National Death Index involves searching national databases containing information about mortality and causes of death. Mortality status was ascertained by computerized matching to national databases and evaluation of the resulting matches. Persons not found to be deceased were assumed alive for analytic purposes.

The NHANES III is preexisting de-identified public use data which do not need specific approval from the National Center for Health Statistics.

We limited our study to individuals 40 years old and older at baseline, the time of their NHANES III interview. All analyses were based on the population estimates generated by applying variables accounting for the design and sampling methodology of the NHANES. The results presented here are generalizable to the noninstitutionalized civilian population of the United States aged 40 and older from 1998-1994.

#### *Previously Diagnosed Diabetes*

The NHANES III assessed participants for diagnosed diabetes using the questions, “Have you ever been told by a doctor that you have diabetes or sugar diabetes?”, “Were you pregnant when you were told that you had diabetes?” and “Other

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3 than during pregnancy, has a doctor ever told you that you have diabetes or sugar  
4 diabetes?" We defined participants as having diagnosed diabetes if they answered "yes"  
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6 to ever having been told they had diabetes, excluding pregnancy. Individuals with  
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8 previously diagnosed diabetes were removed from the analysis. We also removed  
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10 individuals with an HbA1c of 6.5% or greater, to account for undiagnosed diabetes.  
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### 13 *Normoglycemia & Prediabetes*

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15 We defined normoglycemia as an HbA1c level between 4.0%--5.6% (20-38 mmol/mol).  
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17 To control for any potential effect of low HbA1c, we also removed individuals with an  
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19 HbA1c below 4.0% (20 mmol/mol), a level associated with increased all-cause mortality  
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21 in adults without diabetes.<sup>27</sup>  
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27 We defined prediabetes among individuals without previously diagnosed diabetes  
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29 using HbA1c ranges as specified by the American Diabetes Association, 5.7%–6.4% (39-  
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31 46 mmol/mol).<sup>1</sup> This range has been shown in a meta-analysis to be predictive of  
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33 progression to diabetes.<sup>19</sup> We excluded individuals with previously diagnosed diabetes  
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35 because the current glycemic status of those patients may simply represent diabetes  
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37 control. Prediabetes status was missing for 1,637 of the NHANES respondents over the  
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39 age of 40.  
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### 43 *Transferrin Saturation*

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45 Serum iron and total iron-binding capacity (TIBC) were measured in serum, and  
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47 calculated by dividing serum iron by TIBC and multiplying by 100. For the analyses,  
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49 elevated transferrin saturation was categorized as TS  $\geq$ 50%. Individuals with TS below  
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51 25% were removed from the analysis, as low TS has been linked to increased risk of  
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53 mortality.<sup>23</sup> Despite the lack of universal agreement on the upper and lower limits of  
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3 normal TS, these cut points has been used in several studies evaluating diabetes, TS and  
4 mortality.<sup>23,28</sup> Data were missing for transferrin saturation level for 536 of the NHANES  
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6 respondents over the age of 40.  
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### 9 10 *Serum Ferritin*

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12 Serum ferritin was used as a measure of body iron stores and was measured using  
13 the QuantImmune Ferritin IRMA kit. Serum ferritin was categorized for the analyses as  
14 elevated if it was  $\geq 674.1$  pmol/l (300 ng/mL) for males and  $\geq 449.4$  pmol/l (200 ng/mL)  
15 for females.<sup>29</sup> Individuals with serum ferritin below 56.175 pmol/l (25 ng/mL) were  
16 removed from the analysis, as low ferritin has been linked to an increased risk of  
17 mortality.<sup>23</sup> Data were missing for serum ferritin level for 539 of the NHANES  
18 respondents over the age of 40.  
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### 29 *Mortality*

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31 Mortality was measured as all-cause mortality. Mortality status was ascertained  
32 solely by computerized matching to national databases and evaluation of the resulting  
33 matches. All living survey participants examined in this study had been observed for 146  
34 months, and our survival analysis was carried out to December 31, 2006.  
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### 41 *Covariates*

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43 Covariates used in our analyses included: age at baseline in the NHANES III,  
44 gender, race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican-American, and  
45 other), health insurance status, obesity (Body Mass Index computed in the exam of  $\geq 30$ ),  
46 previous diagnosis of a heart attack, previous diagnosis of a stroke, previous diagnosis of  
47 hypertension, previous diagnosis of hypercholesterolemia, previous diagnosis of cancer,  
48 family history of diabetes, family history of myocardial infarction before age 50, and  
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3 current smoking status. Respondents were considered non-smokers if they reported  
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5 smoking less than 100 cigarettes in their life or if they had smoked more than 100  
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7 cigarettes and were not currently smoking.  
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10 In the analysis of serum ferritin, we also controlled for C-reactive protein. Ferritin  
11 is an acute phase reactant as well as an indicator of iron stores and as such may indicate  
12 inflammation. Consequently, we controlled for inflammation by adjusting for C-reactive  
13 protein. C-reactive protein was considered elevated at levels above 3.0 mg/l.<sup>30</sup>  
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### 19 *Analysis*

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21 In an effort to control for potential misclassification of persons who were very ill  
22 at baseline thereby affecting mortality risk of prediabetes, we left-censored the analysis to  
23 exclude any mortality events that occurred in the first three years following the  
24 individuals examination for the first three years of the cohort. Because of the complex  
25 sampling design of the survey, we performed statistical analyses using the statistical  
26 software package SUDAAN (Research Triangle Institute, Raleigh, NC), as recommended  
27 by the National Center for Health Statistics (NCHS). Using SAS (Cary, NC), we  
28 computed Kaplan-Meier plots to show graphically the unadjusted relationship between  
29 all-cause mortality and prediabetes.  
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43 We followed the NCHS recommendations for assessing the reliability of estimates  
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45 than 30% of an estimate it would be considered unreliable All estimates met the criteria  
46 for reliability.  
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52 To accomplish our goals of examining a possible synergistic effect of having  
53 elevated iron with prediabetes we classified the population into 4 groups based upon  
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3 prediabetes or normoglycemia and normal or elevated TS. The population was also  
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5 classified into 4 groups based upon prediabetes or normoglycemia and normal or elevated  
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7 serum ferritin.  
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10 We performed Cox proportional hazards analyses to measure the associations  
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12 between all-cause mortality and prediabetes controlling for all of the studied covariates  
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14 using listwise deletion to account for missing data. In these models, survival time was a  
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16 continuous variable measured in 1-month increments from the date of the exam.  
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19 We also performed adjusted Cox proportional hazards analysis with all-cause  
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21 mortality for prediabetes in the 4 part variables with TS adjusting for the aforementioned  
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23 covariates. For the adjusted Cox proportional hazards analysis with ferritin we adjusted  
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25 for the aforementioned covariates and also C-reactive protein.  
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28 We evaluated the proportionality of the hazards through examination of the  
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30 Schoenfeld residuals.  
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## 33 RESULTS

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35 A total of 8,003 (unweighted) individuals were over 40 years old and had HbA1c  
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37 levels between 4.0 and 6.4%, or 80,653,788 individuals nationally. Baseline  
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39 characteristics for the sample are shown in Table 1. Table 1 indicates that 23.2% of the  
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41 weighted sample had prediabetes, 15.6% of the sample exhibited elevated serum ferritin,  
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43 and 3.3% had elevated TS.  
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47 Of the respondents that had prediabetes, 38.8% died within 12 years (723,702  
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49 died; 11,431,597 survived), compared to 23.4% of respondents with normal HbA1c  
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51 levels (14,527,028 died; 47,458,061 survived). Among individuals with normal TS and  
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53 normoglycemia, 23.1% died (10,724,279 died; 35,649,283 survived), compared to 23.7%  
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3 of those with elevated TS and normoglycemia (412,237 died; 1,327,253 survived), 37.5%  
4  
5 of those with normal TS and prediabetes (5,137,131 died; 8,572,762 survived), and  
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7 44.7% of those with elevated TS and prediabetes (126,633 died; 156,790 survived).  
8  
9 Among individuals with normal ferritin and normoglycemia, 24.3% died (10,967,486  
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11 died; 34,132,718 survived), compared with 38.8% of those with normal ferritin and  
12  
13 prediabetes (5,465,483 died; 8,614,683 survived), 29.2% of those with elevated ferritin  
14  
15 and normoglycemia (2,333,436 died; 5,662,576 survived), and 38.8% of those with  
16  
17 elevated ferritin and prediabetes (1,150,647 died; 1,818,565 survived).  
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22 Table 2 shows the results the adjusted Cox proportional hazards model for  
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24 prediabetes. Table 2 indicates prediabetes alone has a small increased mortality risk. The  
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26 Kaplan Meier curve of the survival and prediabetes over the length of the time under  
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28 observation is shown in Fig 1.  
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32 Table 3 presents results of the analyses combining prediabetes with iron markers.  
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34 In models that examined the impact of a prediabetes state combined with markers of low  
35  
36 iron, the hazard ratios were similar to that of prediabetes alone. However, when  
37  
38 combined with prediabetes, there was an increased mortality risk among individuals with  
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40 TS  $\geq$ 50, as well as with individuals who had increased ferritin. The risk was most  
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42 increased when individuals had elevated ferritin and elevated transferrin saturation  
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44 together. Figure 2 represents the relationship of survival of the four groups over the 12  
45  
46 years under observation. Individuals with prediabetes in the presence of elevated iron  
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48 have lower survival probabilities than other groups. An examination of the Schoenfeld  
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50 residuals suggested proportionality of hazards and appropriateness of the statistical model  
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52 for these analyses.  
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## DISCUSSION

The results of this study in a nationally representative cohort that followed individuals for 12 years confirm that the mortality risk of prediabetes is probably low. This is not unexpected based on the mixed results from previous studies, several of which found either no future mortality risk or risk that was not robust across measures. However, we found that the presence of transferrin saturation and serum ferritin is associated with increased mortality risk of individuals with prediabetes. Among individuals with normal iron levels, those with prediabetes had low mortality risk levels similar to the adjusted risk of prediabetes alone. On the other hand, in adjusted survival analyses, individuals with prediabetes who also had elevated transferrin saturation had substantially increased mortality risk. These findings extend previous work on iron markers and diabetes to the previously uninvestigated area of prediabetes.

These results suggest that additional stratification of individuals with prediabetes on the basis of iron markers would be useful to identify those with higher risk and who might benefit from iron lowering therapies. Previous data has indicated that elevated iron markers are associated with the development of diabetes and that among individuals with diabetes the co-occurrence of elevated TS increases those patients' mortality risk. Early identification of individuals with both conditions (prediabetes, elevated iron) may help in both slowing the development of diabetes as well as decreasing mortality risk. It is important for early identification of these individuals because much like individuals with prediabetes, the vast majority of individuals with elevated iron do not know it.<sup>31</sup> These individuals need to be identified to mitigate the increased risk posed by elevated iron in combination with prediabetes. Such individuals would be targets for intensive

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2  
3 interventions to reduce risk, including typical lifestyle interventions shown to help avoid  
4 the onset of diabetes in people at high risk.<sup>32</sup> Although more research is needed into the  
5 ability of interventions on iron in prediabetes to affect development of diabetes and  
6 mortality risk, some data suggest that reduction of TS improves HbA1c and glucose  
7 control.<sup>33</sup>

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These associations of TS and ferritin with mortality in the context of prediabetes are not surprising especially if elevations of these parameters are interpreted in light of current understanding of iron toxicity.<sup>34</sup> Iron, whether absorbed as iron salts or in dietary heme is processed by enterocytes and released into the plasma where it is transported in a non-reactive state bound to transferrin. Iron that is bound to transferrin is in the  $\text{Fe}^{+3}$  state and is not reactive and, therefore, not toxic. However, when TS is above 40 to 50%, free iron or so-called non-transferrin-bound iron (NTBI) is released into the plasma as the buffering ability of transferrin is exceeded.<sup>35</sup> Labile plasma iron (LPI) is a highly reactive subspecies of NTBI that interacts with hydrogen peroxide through Fenton chemistry to form the extremely powerful oxidants, hydroxyl radical and singlet oxygen. These are the free radicals that ultimately directly damage protein and DNA. Perhaps more importantly, NTBI/LPI species are able to enter cells via ion channels. These channels, unlike the transferrin receptor, are not regulated so this reactive iron freely enters the cytoplasm of the pancreas, pituitary, and heart.

The current results suggest that exposure to excessive free iron is dangerous in the context of prediabetes. Furthermore, elevated ferritin and TS predict poor diabetes control and phlebotomy to reduce iron even over short periods of time improve HbA1c in parallel with changes in TS, even though ferritin is not changed,<sup>33</sup> putting further

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3 emphasis on the fact that NTBI/LPI reflected by TS is the proximal cause of the toxicity.  
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5 Several strategies are available to decrease iron, including chelation therapy and  
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7 phlebotomy. Phlebotomy is an easy, inexpensive, and well-tolerated intervention.  
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10 Reduction in TS by phlebotomy has been shown to improve measures of diabetic  
11  
12 control.<sup>33</sup> Furthermore, correction of severe iron overload can significantly improve  
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14 glucose tolerance.<sup>36</sup> Thus, the finding that a baseline measure of high TS as point  
15  
16 measure of toxic free iron plus elevation of ferritin, evidence of elevated cytosolic iron  
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18 over a longer period of time, predicts increased risk of mortality among individuals with  
19  
20 prediabetes supports the premise that toxic free iron is a health risk.  
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25 The normoglycemic group with elevated iron markers did not show increased  
26  
27 mortality risk in comparison to the reference group of normoglycemic and normal iron  
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29 marker levels. This may seem inconsistent with other data on the increased mortality risk  
30  
31 due to elevated TS by itself. However, there is the potential that the effect of TS on  
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33 mortality is modified by the presence of other variables.<sup>37,38</sup> This effect has been shown in  
34  
35 the past. Rather than being inconsistent with the TS alone and mortality findings, these  
36  
37 new findings enhance our understanding of elevated iron markers and morbidity and  
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39 mortality and allow us to consider the more complex, but real, situation of patients by  
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41 considering multiple variables together rather than independently.  
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46 This study has several limitations. First, although we have a nationally  
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48 representative, population-based cohort followed through the National Death Index, the  
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50 biomarkers are measured only at baseline. There is the possibility that either the  
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52 hyperglycemia or elevated iron measures were identified and interventions were  
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54 implemented to lower these biomarkers. If that were the case and a substantial number of  
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3 individuals did drop their levels due to interventions thereby decreasing the potential  
4 mortality risk, the observed adjusted risk individuals elevated at baseline is even more  
5 concerning. Second, we were only able to follow these individuals for 12 years. It is  
6 possible that this time frame may have been too short to adequately see an effect for a  
7 biomarker like prediabetes. However, we did censor the first three years of mortality so  
8 that any deaths in that time frame would not be attributed to prediabetes. The model still  
9 found a substantial mortality risk for the prediabetes plus iron markers in this length of  
10 time. Third, we were unable to evaluate the relationship between being elevated on both  
11 transferrin saturation and serum ferritin with prediabetes on the risk of mortality. We  
12 attempted such an analysis but the number of individuals in the group with prediabetes  
13 and elevation on both iron markers was small and the population estimates were deemed  
14 unreliable.

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32 In conclusion, this study representative of the population of the United States  
33 helps to clarify the current evidence on the mortality risk of prediabetes and provides  
34 further support for the role of elevated iron markers in health risk. Future screening and  
35 intervention programs for prediabetes may benefit from additional strategies to recognize  
36 and treat iron elevations, particularly transferrin saturation.  
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## CONTRIBUTION STATEMENT

AGM: conception, design, analysis, and drafting of manuscript

RJT: design, analysis, and drafting of manuscript

TDC: conception, design, and drafting of manuscript

RB: design and analysis

## COMPETING INTERESTS

The authors report no competing interests.

**REFERENCES**

1. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 2012;35:S64–S71.
2. Bullard KM, Saydah SH, Imperatore G, et al. Secular changes in U.S. Prediabetes prevalence defined by hemoglobin A1c and fasting plasma glucose: National Health and Nutrition Examination Surveys, 1999-2010. *Diabetes Care* 2013;36:2286–93.
3. Mainous AG 3rd, Tanner RJ, Baker R, et al. Prevalence of prediabetes in England from 2003 to 2011: population-based, cross-sectional study. *BMJ Open* 2014 Jun 9;4(6):e005002. doi: 10.1136/bmjopen-2014-005002. Accessed 29 June, 2014.
4. Centers for Disease Control and Prevention (CDC). Awareness of prediabetes--United States, 2005-2010. *MMWR Morb Mortal Wkly Rep*. 2013 Mar 22;62:209-12.
5. de Vegt F, Dekker JM, Ruhé HG, et al. Hyperglycaemia is associated with all-cause and cardiovascular mortality in the Hoorn population: the Hoorn Study. *Diabetologia* 1999;42:926–31.
6. Fuller JH, Shipley MJ, Rose G, et al. Coronary-heart-disease risk and impaired glucose tolerance. The Whitehall study. *Lancet* 1980;1:1373–76.
7. Valdés S, Botas P, Delgado E, Cadórniga FD. Mortality risk in Spanish adults with diagnosed diabetes, undiagnosed diabetes, or pre-diabetes. The asturias study 1998-2004. *Rev Esp Cardiol (Engl Ed)* 2009;62:528–34.
8. Deedwania P, Patel K, Fonarow GC, et al. Prediabetes is not an independent risk factor for incident heart failure, other cardiovascular events or mortality in older adults: findings from a population-based cohort study. *Int J Cardiol* 2013;168:3616–22.

- 1  
2  
3 9. Kowall B, Rathmann W, Heier M, et al. Categories of glucose tolerance and  
4  
5 continuous glycemic measures and mortality. *Eur J Epidemiol* 2011;26:637–45.  
6  
7
- 8 10. Zhou XH, Qiao Q, Zethelius B, et al. Diabetes, prediabetes and cancer mortality.  
9  
10 *Diabetologia* 2010;53:1867–76.  
11
- 12 11. Barr EL, Zimmet PZ, Welborn TA, et al. Risk of cardiovascular and all-cause  
13  
14 mortality in individuals with diabetes mellitus, impaired fasting glucose, and impaired  
15  
16 glucose tolerance: the Australian Diabetes, Obesity, and Lifestyle Study (AusDiab).  
17  
18 *Circulation* 2007;116:151–57.  
19
- 20 21 12. Orban E, Schwab S, Thorand B, Huth C. Association of iron indices and type 2  
22  
23 diabetes: a meta-analysis of observational studies. *Diabetes Metab Res Rev* 2014;30:372–  
24  
25 94.  
26  
27
- 28 29 13. Bao W, Rong Y, Rong S, Liu L. Dietary iron intake, body iron stores, and the risk of  
30  
31 type 2 diabetes: a systematic review and meta-analysis. *BMC Med* 2012;10:119. doi:  
32  
33 10.1186/1741-7015-10-119. Accessed 8 July, 2014.  
34  
35
- 36 37 14. Ellervik C, Mandrup-Poulsen T, Tybjærg-Hansen A, Nordestgaard BG. Total and  
38  
39 cause-specific mortality by elevated transferrin saturation and hemochromatosis genotype  
40  
41 in individuals with diabetes: two general population studies. *Diabetes Care* 2014;37:444–  
42  
43 52.  
44
- 45 46 15. Masuda Y, Ichii H, Vaziri ND. At pharmacologically relevant concentrations  
47  
48 intravenous iron preparations cause pancreatic beta cell death. *Am J Transl Res*.  
49  
50 2013;6:64-70.  
51  
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60
16. Ellervik C, Tybjaerg-Hansen A, Nordestgaard BG. Total mortality by transferrin saturation levels: Two general population studies and a meta analysis. *Clin Chem* 2011;57:459–466.
17. Kim KS, Son HG, Hong NS, Lee DH. Associations of serum ferritin and transferrin % saturation with all-cause, cancer, and cardiovascular disease mortality: Third National Health and Nutrition Examination Survey follow-up study. *J Prev Med Public Health* 2012;45:196-203.
18. Mainous AG 3rd, Gill JM, Carek PJ. Elevated transferrin saturation and mortality. *Ann Fam Med* 2004;2:133–138.
19. Mainous AG 3rd, Wells B, Carek PJ, et al. The mortality risk of elevated serum transferrin saturation and consumption of dietary iron. *Ann Fam Med* 2004;2:139-44.
20. Stack AG, Mutwali AI, Nguyen HT, et al. Transferrin saturation ratio and risk of total and cardiovascular mortality in the general population. *QJM*. 2014;107:623-33.
21. Wells BJ, Mainous AG 3rd, King DE, et al. The combined effect of transferrin saturation and low density lipoprotein on mortality. *Fam Med* 2004;36:324-9.
22. Ellervik C, Marott JL, Tybjærg-Hansen A, Schnohr P, Nordestgaard BG. Total and Cause-Specific Mortality by Moderately and Markedly Increased Ferritin Concentrations: General Population Study and Metaanalysis. *Clin Chem*. 2014 Aug 25. pii: clinchem.2014.229013.
23. Ponikowska B, Suchocki T, Paleczny B, et al. Iron status and survival in diabetic patients with coronary artery disease. *Diabetes Care* 2013;36:4147–56.

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24. Morris DH, Khunti K, Achana F, et al. Progression rates from HbA(1c) 6.0-6.4% and other prediabetes definitions to type 2 diabetes: a meta-analysis. *Diabetologia* 2013;56:1489–93.
25. National Center for Health Statistics. Plan and operation of the Third National Health and Nutrition Examination Survey, 1988-1994. *Vital Health Stat.* 1994;1(32).  
[http://www.cdc.gov/nchs/data/series/sr\\_01/sr01\\_032.pdf](http://www.cdc.gov/nchs/data/series/sr_01/sr01_032.pdf). Accessed 15 October 2014.
26. National Center for Health Statistics. Office of Analysis and Epidemiology, NCHS 2011 Linked Mortality Files Matching Methodology, September, 2013. Hyattsville, Maryland.  
[http://www.cdc.gov/nchs/data/datalinkage/2011\\_linked\\_mortality\\_file\\_matching\\_methodology.pdf](http://www.cdc.gov/nchs/data/datalinkage/2011_linked_mortality_file_matching_methodology.pdf). Accessed 20 August, 2014.
27. Carson AP1, Fox CS, McGuire DK, Levitan EB, Laclaustra M, Mann DM, Muntner P. Low hemoglobin A1c and risk of all-cause mortality among US adults without diabetes. *Circ Cardiovasc Qual Outcomes.* 2010 Nov;3(6):661-7.
28. Ellervik C, Andersen HU, Tybjærg-Hansen A, et al. Total mortality by elevated transferrin saturation in patients with diabetes. *Diabetes Care* 2013;36:2646–54.
29. Adams PC, Reboussin DM, Barton JC, et al. Hemochromatosis and Iron Overload Screening (HEIRS) Study Research Investigators. Hemochromatosis and iron-overload screening in a racially diverse population. *N Engl J Med.* 2005;352:1769-78.
30. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice. A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. *Circulation.* 2003;107(3):499 – 511.

- 1  
2  
3  
4 31. Mainous AG 3rd, Wright RU, Hulihan MM, et al. Elevated transferrin saturation,  
5 health-related quality of life and telomere length. *Biometals* 2014;27:135–41  
6  
7  
8 32. Portero McLellan KC, Wyne K, Villagomez ET, Hsueh WA. Therapeutic  
9 interventions to reduce the risk of progression from prediabetes to type 2 diabetes  
10 mellitus. *Ther Clin Risk Manag* 2014;10:173–88.  
11  
12  
13 33. Fernández-Real JM, Peñarroja G, Castro A, García-Bragado F, Hernández-Aguado I,  
14 Ricart W. Blood letting in high-ferritin type 2 diabetes: effects on insulin sensitivity and  
15 beta-cell function. *Diabetes* 2002;51:1000–4.  
16  
17  
18 34. Hansen JB, Moen IW, Mandrup-Poulsen T. Iron: the hard player in diabetes  
19 pathophysiology. *Acta Physiol* 2014;210:717–32.  
20  
21  
22 35. Coates TD. Physiology and pathophysiology of iron in hemoglobin-associated  
23 diseases. *Free Radic Biol Med* 2014;72C:23–40.  
24  
25  
26 36. Farmaki K, Tzoumari I, Pappa C, et al. Normalisation of total body iron load with  
27 very intensive combined chelation reverses cardiac and endocrine complications of  
28 thalassaemia major. *Br J Haematol* 2010;148:466–75.  
29  
30  
31 37. Mainous AG 3rd, Wells B, Carek PJ, Gill JM, Geesey ME. The mortality risk of  
32 elevated serum transferrin saturation and consumption of dietary iron. *Ann Fam Med*.  
33 2004;2:139-44.  
34  
35  
36 38. Wells BJ, Mainous AG 3rd, King DE, Gill JM, Carek PJ, Geesey ME. The combined  
37 effect of transferrin saturation and low density lipoprotein on mortality. *Fam Med*.  
38 2004;36(5):324-9.  
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Table 1: Baseline Characteristics of the Sample

	<b>Study Sample (%)</b>
<b>Unweighted Sample Size</b>	8,003
<b>Population Estimate</b>	80,653,788
<b>Age, years</b>	
40-54	49.6
55-69	31.2
70+	19.2
<b>Sex, male</b>	46.1
<b>Race/Ethnicity</b>	
Non-Hispanic White	82.2
Non-Hispanic Black	8.3
Mexican-American	3.2
Other	6.4
<b>Has health insurance</b>	93.6
<b>Obese</b>	23.3
<b>Current Smoker</b>	23.6
<b>Has diagnosed high cholesterol</b>	40.1
<b>Has diagnosed hypertension</b>	31.3
<b>Ever diagnosed with heart attack</b>	5.3
<b>Ever diagnosed with stroke</b>	2.8
<b>Ever diagnosed with cancer</b>	12.3
<b>Relative with diabetes</b>	41.2
<b>Relative with heart attack before age 50</b>	15.4
<b>Elevated C-reactive protein</b>	1.2
<b>Elevated Transferrin Saturation</b>	3.3
<b>Elevated Ferritin</b>	15.6
<b>Prediabetes</b>	23.2
<b>Assumed deceased</b>	27.0

Table 2: Adjusted Hazard Ratios from Cox Regression for Mortality Risk of Individuals with Prediabetes

	<b>Hazard Ratio</b>	<b>95% Confidence Interval</b>
<b>Adjusted Model<sup>a</sup></b>		
Prediabetes	1.04	1.00-1.08
Normoglycemia	1.0	--

<sup>a</sup>Adjusted for: age, gender, race/ethnicity, health insurance status, obesity, current smoking status, diagnosed heart attack, diagnosed stroke, diagnosed hypertension, diagnosed high cholesterol, diagnosis of cancer, family history of diabetes, and family history of early heart attack.

Table 3: Adjusted Hazard Ratios from Cox Regression for Mortality Risk of Individuals with Prediabetes and Iron Markers

	Hazard Ratio	95% Confidence Interval
<b>Adjusted Model<sup>a</sup></b>		
Normal Transferrin Saturation/ Normoglycemia	1.00	--
Normal Transferrin Saturation/ Prediabetes	1.13	1.08-1.19
Elevated Transferrin Saturation/ Normoglycemia	.98	.90-1.07
Elevated Transferrin Saturation/ Prediabetes	1.88	1.06-3.30
<b>Adjusted Model<sup>b</sup></b>		
Normal Ferritin/ Normoglycemia	1.00	--
Normal Ferritin/ Prediabetes	1.15	1.09-1.20
Elevated Ferritin/ Normoglycemia	1.05	1.00-1.12
Elevated Ferritin/ Prediabetes	1.14	1.04-1.24

<sup>a</sup>Adjusted for: age, gender, race/ethnicity, health insurance status, obesity, current smoking status, diagnosed heart attack, diagnosed stroke, diagnosed hypertension, diagnosed high cholesterol, diagnosis of cancer, family history of diabetes, and family history of early heart attack.

<sup>b</sup>Adjusted for: age, gender, race/ethnicity, health insurance status, obesity, current smoking status, diagnosed heart attack, diagnosed stroke, diagnosed hypertension, diagnosed high cholesterol, diagnosis of cancer, family history of diabetes, family history of early heart attack, and elevated C-reactive protein.

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3 Figure 1. Kaplan Meier curve of survival among individuals with prediabetes or normal  
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6 glycaemic levels.

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8 ——— Normoglycemia

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10 ——— Prediabetes  
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15 Figure 2. Kaplan Meier curve of survival among individuals with prediabetes and  
16  
17 elevated transferrin saturation

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20 ——— Normoglycemia and Normal Transferrin Saturation

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22 ——— Normoglycemia and Elevated Transferrin Saturation

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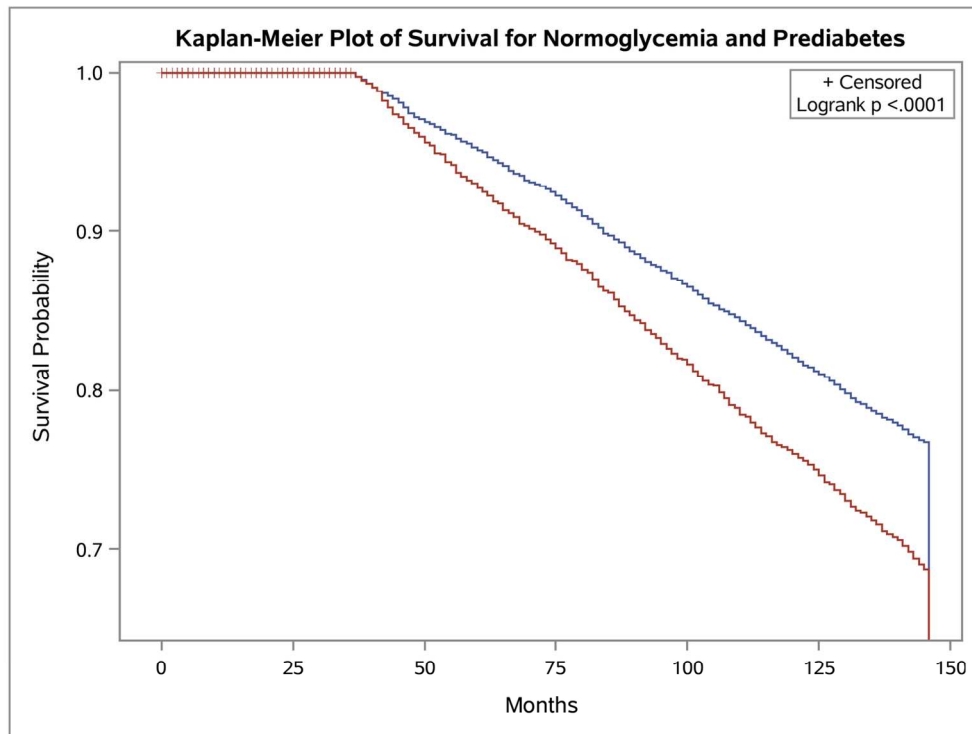
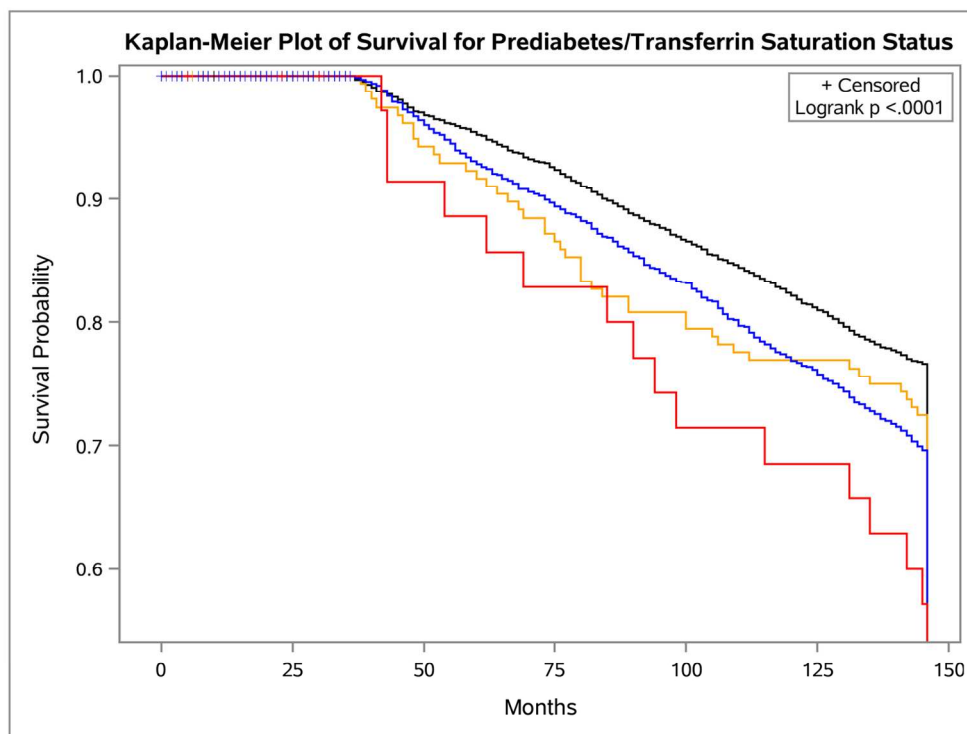


Figure 1-1-1. Kaplan Meier curve of survival among individuals with prediabetes or normal glyceimic levels.

blue line = Normoglycemia  
 red line = Prediabetes  
 131x100mm (300 x 300 DPI)

view only





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Figure 2. Kaplan Meier curve of survival among individuals with prediabetes and elevated transferrin saturation

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black line = Normoglycemia and Normal Transferrin Saturation  
yellow line = Normoglycemia and Elevated Transferrin Saturation  
blue line = Prediabetes and Normal Transferrin Saturation  
red line = Prediabetes and Elevated Transferrin Saturation  
131x99mm (300 x 300 DPI)

## STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page number
<b>Title and abstract</b>	1	(a) Indicate the study's design with a commonly used term in the title or the abstract	1
		(b) Provide in the abstract an informative and balanced summary of what was done and what was found	2
<b>Introduction</b>			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any prespecified hypotheses	4-5
<b>Methods</b>			
Study design	4	Present key elements of study design early in the paper	5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	5
Participants	6	(a) <i>Cohort study</i> —Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up <i>Case-control study</i> —Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls <i>Cross-sectional study</i> —Give the eligibility criteria, and the sources and methods of selection of participants	5
		(b) <i>Cohort study</i> —For matched studies, give matching criteria and number of exposed and unexposed <i>Case-control study</i> —For matched studies, give matching criteria and the number of controls per case	NA
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	6-8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	6-8
Bias	9	Describe any efforts to address potential sources of bias	9
Study size	10	Explain how the study size was arrived at	5-8
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5-8
Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding	8-9
		(b) Describe any methods used to examine subgroups and interactions	8
		(c) Explain how missing data were addressed	8
		(d) <i>Cohort study</i> —If applicable, explain how loss to follow-up was addressed <i>Case-control study</i> —If applicable, explain how matching of cases and controls was addressed <i>Cross-sectional study</i> —If applicable, describe analytical methods taking account of sampling strategy	NA
		(e) Describe any sensitivity analyses	NA

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<b>Results</b>			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5, 9
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9, 19
		(b) Indicate number of participants with missing data for each variable of interest	6-7
		(c) <i>Cohort study</i> —Summarise follow-up time (eg, average and total amount)	5
Outcome data	15*	<i>Cohort study</i> —Report numbers of outcome events or summary measures over time	19
		<i>Case-control study</i> —Report numbers in each exposure category, or summary measures of exposure	
		<i>Cross-sectional study</i> —Report numbers of outcome events or summary measures	
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9-10 20- 22
		(b) Report category boundaries when continuous variables were categorized	NA
		(c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	10
<b>Discussion</b>			
Key results	18	Summarise key results with reference to study objectives	10- 11
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	12- 13
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12
Generalisability	21	Discuss the generalisability (external validity) of the study results	13
<b>Other information</b>			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	14

\*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

**Note:** An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at <http://www.plosmedicine.org/>, Annals of Internal Medicine at <http://www.annals.org/>, and Epidemiology at <http://www.epidem.com/>). Information on the STROBE Initiative is available at [www.strobe-statement.org](http://www.strobe-statement.org).