Title

Higher ferritin levels, but not serum iron or transferrin saturation, are associated with Type 2 diabetes mellitus in adult men and women free of genetic haemochromatosis.

Short title

Ferritin and diabetes risk

Authors

Bu B. Yeap[1,2], Mark L. Divitini[3], Jenny E. Gunton[4], John K. Olynyk[5,6,7], John P. Beilby[8], Brendan McQuillan[1], Joseph Hung[1], Matthew W. Knuiman[3]

Institutions

[1]School of Medicine and Pharmacology, University of Western Australia, Perth, Western Australia, [2]Department of Endocrinology and Diabetes, Fremantle Hospital, Fremantle, Western Australia, [3]School of Population Health, University of Western Australia, Perth, Western Australia, [4]Westmead Hospital, University of Sydney and Garvan Institute of Medical Research, Sydney, New South Wales, [5]Department of Gastroenterology, Fremantle Hospital, Fremantle, Western Australia, [6]School of Biomedical Sciences, Curtin University, Bentley, Western Australia, [7]Institute for Immunology and Infectious Diseases, Murdoch University, Murdoch, Western Australia, [8]PathWest Laboratory Medicine, Sir Charles Gairdner Hospital, Perth, Western Australia, Australia.

Correspondence

Bu Beng Yeap MBBS, PhD

Professor, School of Medicine and Pharmacology,

Level 2, T Block, Fremantle Hospital, Alma Street, Fremantle, WA 6160,

Australia. Tel: +61 8 9431 3229

Fax: +61 8 9431 2977

Email: bu.yeap@uwa.edu.au

Key words

Ferritin, iron, transferrin, diabetes mellitus

Acknowledgements

We thank the staff of the Busselton Health Survey and the Data Linkage Unit, Health Department of Western Australia for their assistance with the study, and the staff of PathWest Laboratory Medicine, Sir Charles Gairdner Hospital for excellent technical assistance. We especially thank all the men and women who took part in the Busselton Health Survey, Western Australia.

Disclosures

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Manuscript word count 2,932

Tables 3

Abstract

Context

Iron overload predisposes to diabetes, and higher ferritin levels have been associated with diabetes. However, it is unclear whether ferritin reflects differences in iron-related parameters between diabetic and non-diabetic persons. We examined associations of serum ferritin, iron and transferrin saturation with Type 2 diabetes in adults without genetic predisposition to iron overload.

Design, participants and measurements

Cross-sectional analysis of community-dwelling men and women aged 17-97 years from the Busselton Health Survey, Western Australia. Men and women carrying genotypes associated with haemochromatosis (C282Y/C282Y or C282Y/H63D) were excluded. Serum ferritin, iron and transferrin saturation were assayed.

Results

There were 1,834 men (122 with diabetes, 6.6%) and 2,351 women (141 with diabetes, 6.0%). In men, higher serum ferritin was associated with diabetes after adjusting for age, smoking, alcohol, cardiovascular history, BMI, waist, blood pressure, lipids, CRP, adiponectin, ALT and GGT (odds ratio [OR]: 1.29 per 1 unit increase log ferritin, 95% confidence interval [CI]=1.01-1.65, p=0.043). In women, higher serum ferritin was associated with diabetes (fully-adjusted OR: 1.31 per 1 unit increase log ferritin, 95% CI=1.04-1.63, p=0.020; 1.84 for tertile (T)3 vs T1, 95% CI=1.09-3.11). Neither iron levels nor transferrin saturation were associated with diabetes risk in men or women. Higher ferritin was not associated with insulin resistance in non-diabetic adults.

Conclusions

In adults, higher ferritin levels are independently associated with prevalent diabetes while iron and transferrin saturation are not. Ferritin is a robust biomarker for diabetes risk, but

further investigation is needed to clarify whether this relationship is mediated via iron metabolism.

Introduction

Diabetes mellitus is a recognised complication of excessive iron accumulation due to increased absorption and retention of dietary iron in the setting of hereditary haemochromatosis, and transfusion-dependent iron overload [1,2]. Proposed underlying mechanisms include roles for iron accumulation in both beta cell failure and insulin resistance, with insulin resistance being more relevant to diabetes risk in the setting of transfusion or diet-related iron excess [2]. Serum ferritin levels are commonly utilised as a marker of body iron stores, and both dietary iron intake and higher ferritin levels predict incidence of Type 2 diabetes in epidiemological studies [3-5]. Conversely, iron restriction improves glycemia in diabetic rats [6] and phlebotomy reduces HbA1c levels in patients with Type 2 diabetes who have high ferritin levels [7]. However it remains unclear whether higher ferritin levels are a manifestation of excessive body iron stores which interfere directly with glucose metabolism; or represent a marker of systemic processes such as inflammation which correlate to diabetes risk [2].

In prospective studies, dietary iron intake in the form of heme from meat products and serum ferritin levels predict incidence of Type 2 diabetes but these associations are attenuated by adjustment for markers of inflammation [8]. Furthermore, there is doubt over whether a dose-response effect exists with a non-linear association being reported in a recent meta-analysis [9]. Recently, iron has been found to negatively regulate adiponectin, an insulin-sensitising adipokine, suggesting that adiponectin may mediate the relationship between iron and glucose metabolism [10]. To clarify whether or not ferritin and other markers of iron

metabolism are associated independently with diabetes risk, we analysed the association of serum ferritin, iron and transferrin with diabetes risk with adjustment for conventional risk factors, the inflammatory marker C-reactive protein (CRP) and adiponectin in a large cohort of community-dwelling adult men and women.

Participants and Methods

Study population

Busselton is a coastal town in Western Australia, whose residents have participated in a series of health surveys from 1966 onwards. In 1994-1995, all surviving subjects of earlier Busselton surveys were invited to participate in a follow-up survey [11]. More than 90% of the Busselton population is of Caucasian ethnic origin. The present study is based on 4,185 men and women aged 19-97 years who attended the 1994/95 survey and provided blood samples for analysis. The study was approved by the Human Research Ethics Committee of the University of Western Australia, and all participants provided written informed consent.

Assessment of medical comorbidities

Methods used in the Busselton Health Survey have been described previously [11]. Participants were asked to complete a comprehensive health and lifestyle questionnaire and underwent a physical assessment. Blood pressure was measured using a mercury sphygmomanometer after five minutes at rest in a sitting position. Height was measured using a stadiometer and weight in light underclothes. Smoking, diabetes, and the use of antihypertensive or glucose-lowering medications were obtained via questionnaire. Participants were defined as having diabetes if they reported being told by a doctor that they had diabetes, and/or had a fasting blood glucose ≥7.0 mmol/L, or were taking glucose-lowering medications. Participants were considered to have Type 2 diabetes unless they (i)

reported age of onset <40 years and were commenced on insulin at diagnosis, or (ii) had age of onset between 40-60 years, were commenced on insulin from the time of diagnosis, were on insulin at recruitment and had body mass index (BMI) <30 kg/m^2 [12]. Other medical history was assessed from questionnaire responses and also via the Western Australian Data Linkage System (WADLS), which captures electronically all admissions to public or private hospitals in Western Australia [13]. A history of coronary heart disease (CHD) or stroke was defined as any hospital admission with a primary or secondary diagnosis of CHD or stroke during the preceding 15 years prior to the survey.

Laboratory assays

Blood samples were collected in the early morning after an overnight fast. Serum ferritin, iron and transferrin saturation, and mutations in the *HFE* gene were assessed as previously reported [14]. Briefly, serum ferritin levels were measured by chemiluminescence immunoassay (ACS-180, Chiron Diagnostics, Norwood, Massachusetts, USA). Serum iron levels were measured by a standard colorimetric method and serum transferrin levels by immunoturbidimetry on an automated analyser (Model 917, Hitachi, Toyko, Japan). Serum transferrin saturation values were calculated as follows: ([serum iron/2]*serum transferrin)*100. DNA was extracted from whole blood and PCR analysis performed to identify C282Y and H63D mutations of the *HFE* gene. Other biochemical analyses were performed as previously described [15]. Briefly, fasting serum cholesterol, HDL and triglycerides were determined at the time of the survey by standard enzymatic methods (Hitachi 747 analyser, Roche Diagnostics, Australia). Glucose was measured using a hexokinase assay. Insulin was assayed using a Tosoh A1A-600 immunoassay analyzer using a two-site immunoenzymometric assay. We measured C-reactive protein (CRP) using a particle-enhanced immunoturbidimetric assay on a Modular analyser (Roche Diagnostics,

Germany). Plasma adiponectin levels were measured by a commercially available quantitative sandwich enzyme immunoassay technique (R&D Systems Inc., Minneapolis, Minnesota, USA). We estimated insulin resistance using homeostatic models (HOMA-R) as previously described [16]. Adults with glucose ≤3.5 or >25 mmol/L were excluded. HOMA-R was calculated as (insulin*glucose)/22.5 and was log transformed for analyses.

Statistical analyses

Descriptive statistics are presented as mean and standard deviation (SD) or as percentages (Table 1). Logistic regression models were used to examine the relationships between serum ferritin, iron and transferrin saturation as continuous variables and categorised into tertiles with the presence of diabetes in men and women (Tables 2-4). Models were adjusted sequentially for variables that could potentially modify their association with prevalent diabetes. Models were age-adjusted, then additionally adjusted for smoking, alcohol consumption, CHD or stroke history, BMI, waist, systolic and diastolic blood pressure, high-density lipoprotein cholesterol (HDL), triglycerides, CRP, adiponectin, alanine transaminase (ALT) and gamma-glutamyl transpeptidase (GGT). Variables were log transformed where the original distributions were skewed. To identify the important and independent correlates of (log) HOMA-R in non-diabetic men and women we fitted a multivariable model (Table 5). A p value of <0.05 or 95% confidence intervals that did not cross 1.0 were regarded as significant.

Results

Characteristics of the study population

The age ranges for men and women were 16.9 to 97.3 and 16.5 to 92.2 years respectively. We excluded 22 adults with Type 1 diabetes and 69 who carried either of the C282Y/C282Y

or C282Y/H63D genotypes associated with hereditary haemochromatosis. This left 1,834 men (122 with Type 2 diabetes, 6.6%) and 2,351 women (141 with Type 2 diabetes, 6.0%) for analysis. Characteristics of the study cohort stratified by gender and the presence or absence of diabetes are shown (Table 1). Serum ferritin, iron and transferrin saturation are shown without and with log transformation and as tertiles. Men with diabetes were older, more likely to have smoked and less likely to be current drinkers. In men, diabetes was associated with a history of cardiovascular disease, higher BMI and waist circumference, systolic and diastolic blood pressure, triglycerides, fasting glucose, insulin, HOMA-R, GGT and CRP, but with lower HDL. These cardiometabolic variables were similarly associated with diabetes in women with the exceptions of smoking and diastolic blood pressure, as was menopausal status. Ferritin levels were significantly higher in men and women with diabetes, when examined as both continuous variables and in tertiles. Serum iron was not associated with diabetes in either men or women. Lower transferrin saturation was associated with diabetes when analysed as a log transformed variable or in tertiles in men but not in women.

TABLE 1

Association of ferritin with prevalent diabetes

In men, ferritin was significantly associated with diabetes when examined as a continuous variable and in tertile groups after adjustment for age alone (Table 2). The association of ferritin as a continuous variable with diabetes remained significant in further models that adjusted for all other cardiometabolic risk factors including CRP, adiponectin, ALT and GGT with an odds ratio (OR) of 1.29 per 1 unit increase log ferritin (95% confidence interval [CI]=1.01-1.65). In women, there was also a significant association between ferritin and diabetes (Table 2). The fully-adjusted OR for diabetes was 1.31 per 1 unit increase log

ferritin (95% CI=1.04-1.63); and 1.84 (95% CI=1.09-3.11) for women with ferritin in the highest tertile (T3) of values compared to the lowest (T1).

TABLE 2

Regression analysis of serum iron and prevalent diabetes

In men and women, serum iron was not significantly associated with diabetes when examined either as a continuous variable or in tertile groups. The fully-adjusted OR for men was 0.90 (95% CI=0.47-1.72) for a 1 unit increase in log ferritin and 0.80 (95% CI=0.49-1.33) for T3 vs T1. For women the fully-adjusted OR were 1.08 (95% CI=0.64-1.83) and 1.21 (95% CI=0.73-1.99) respectively.

Regression analysis of transferrin saturation and prevalent diabetes

Of the 1,834 men and 2,351 women analysed, there were 57 men (6 diabetic and 51 non-diabetic) and 29 women (1 diabetic and 28 non-diabetic) with transferrin saturation >50%. In men, there was some evidence of a negative association between transferrin saturation and diabetes after adjustment for age alone. The age-adjusted OR of diabetes was 0.51 (95% CI=0.31-0.84, p=0.029 for between groups comparison) for transferrin saturation in T3 vs T1, but this was attenuated to 0.57 (95% CI=0.34-0.96, p=0.108 for between groups comparison) after full adjustment for other risk factors. Transferrin saturation as a continuous variable was not associated with diabetes in men (fully-adjusted OR 0.83, 95% CI=0.49-1.43). In women, there was no significant association of transferrin saturation with prevalent diabetes (fully-adjusted OR 1.11, 95% CI=0.71-1.74 for continuous variable and 1.26, 95% CI=0.80-1.99 for T3 vs T1).

Multivariable analysis of factors associated with insulin resistance

The fitted multivariable model examining factors associated with insulin resistance as estimated using HOMA-R is shown for men and women without diabetes (Table 5). In men, age was negatively associated with HOMA-R, as were HDL. CHD or stroke history, BMI, waist circumference, systolic blood pressure, triglycerides, CRP and ALT were positively associated with HOMA-R in men. In women, age, use of hormone replacement therapy in post-menopausal women, and HDL were negatively associated with HOMA-R. BMI, waist circumference, systolic blood pressure, triglycerides and GGT were positively associated. Adiponectin was inversely associated with HOMA-R in both men and women. Ferritin was not associated with HOMA-R in either men or women without diabetes.

TABLE 5

Discussion

Higher serum ferritin levels are independently associated with diabetes in men and women, both in age-adjusted and in multivariable adjusted models accounting for cardiometabolic factors. This association is present for ferritin analysed as a continuous variable, and in women for ferritin levels in the highest tertile of values. However neither serum iron nor transferrin saturation were associated with diabetes in this cohort. Furthermore, ferritin was not associated with insulin resistance in men or women without diabetes.

Our results are consistent with previous studies relating serum ferritin levels to diabetes risk [3-5,17-19]. However, higher ferritin levels are also associated with obesity and the metabolic syndrome [20,21] and with cardiovascular risk [22]. There is also a recognised association between ferritin levels and the presence and severity of fatty liver disease [23].

We found that the association of serum ferritin with prevalent diabetes was largely unchanged after adjustment for cardiometabolic risk factors including adiponectin, and it remained significant after adjustment for ALT and GGT. Therefore, our results suggest that the relationship between ferritin level and diabetes risk is not modulated by the presence of obesity or by common risk factors for cardiovascular disease and diabetes. Furthermore, in our analysis ferritin was not independently associated with insulin resistance as assessed using HOMA-R in men or women without diabetes. This contrasts with previous reports implicating ferritin levels with insulin resistance syndromes and with hyperinsulinaemia [20,21,24,25]. Therefore, while the association between higher ferritin and diabetes is robust, the mechanisms by which ferritin might contribute to diabetes risk remains unclear.

We measured serum iron and transferrin saturation to provide additional indicators of body iron stores [26]. Transferrin serves as the major vehicle for iron delivery into cells via the transferrin receptor, with increased saturation reflective of higher body iron stores [26]. Previous studies have indicated that transferrin saturation ≥50% is associated with increased risk of diabetes [27,28]. However, we found no association of either serum iron or transferrin saturation with diabetes in men or women, with the large majority of our study participants having transferrin saturation below this threshold. By contrast, there was a trend for higher transferrin saturation to be associated with lower risk of diabetes in men, although this was not significant in the fully adjusted models. Nevertheless, the trend is in the opposite direction from that expected should higher body iron stores contribute to diabetes risk. We found no evidence to support the concept that iron availability as reflected by serum iron or transferrin saturation contributes to diabetes risk. Previous epidemiological studies have shown associations of not only ferritin levels [3-5] but also dietary heme iron intake with risk of Type 2 diabetes [3,8,9]. We did not have data on dietary intake in our cohort however our

examination of iron status included serum iron and transferrin saturation in addition to ferritin. The discordance between associations of ferritin with Type 2 diabetes and the absence of any association of serum iron or transferrin saturation are consistent with ferritin being a marker of metabolic risk distinct from iron metabolism.

In addition to being an indicator of body iron stores, circulating ferritin is an acute phase reactant which like CRP is elevated in inflammatory conditions, while transferrin is not [29]. Inflammation is a mediator of diabetes risk and higher levels of CRP are associated with impaired glucose tolerance and diabetes [30,31]. Adiponectin is has also been proposed as a mediator of inflammatory responses and insulin sensitivity [32]. However, adjustment for CRP and for adiponectin in the final multivariate model did not alter the association of ferritin with diabetes in men and women. Therefore, measurement of ferritin captures a key determinant of diabetes risk that is not achieved via measurement of either CRP, adiponectin or both. Ferritin is a robust biomarker for diabetes in our study population, but the negative associations of iron and transferrin saturation with diabetes lead us to postulate that mechanisms independent of body iron status are involved.

Strengths of our study are the large size of the cohort, its inclusion of men and women spanning younger, middle and older ages, extensive phenotyping and availability of physical, metabolic and laboratory variables for the analyses. As this cohort had previously been studied to evaluate the clinical expression of hereditary haemochromatosis [14], genotyping data were available and we were able to exclude men and women with C282Y and H63D polymorphisms associated with this disease. We compared associations not only of ferritin but also serum iron and transferrin saturation, we conducted analyses adjusting for a wide range of potential confounders and explored the influence of CRP and adiponectin. We

acknowledge several limitations of this study. This is an observational study and we performed a cross-sectional analysis thus we cannot infer causation. The men and women were community-dwelling survivors from previous surveys, thus a "healthy survivor" effect might be present. We did not have serial blood samples to assess longitudinal changes in the levels of ferritin, iron and transferrin instead we used a single blood sample albeit collected early in the morning in the fasted state. While HOMA-R is a useful tool for epidiemiological studies, it may not estimate insulin resistance as accurately as more invasive methods [16]. Additionally the Busselton cohort is almost entirely Caucasian in ethnicity thus care must be taken before extrapolating our findings to other populations. All participants in the study were community-dwelling adults who volunteered to attend a study centre for assessment, making it less likely that they were unwell with intercurrent illness at the time of blood sampling.

Smaller studies have reported associations of higher ferritin with HOMA-R and with metabolic syndrome in both men and women [21,25,33]. One study of non-diabetic adults comprising 140 men and 277 women showed a correlation between ferritin level and HOMA-R in women but not in men [34]. Given the size of our study population and the coefficients demonstrated, any clinically or statistically significant influence of ferritin on HOMA-R in men is unlikely to be present. There was no statistically significant association of ferritin with HOMA-R in women in our cohort.

There are limited data documenting effects of venesection on diabetes risk. One study of 28 patients with Type 2 diabetes reported improved insulin sensitivity and lower HbA1c after venesection [7]. Another in 64 adults with metabolic syndrome showed no difference in HOMA-R in control and phlebotomy groups [35]. Therefore additional studies are needed to

test whether manipulation of ferritin levels whether by phlebotomy, iron chelation therapy or dietary iron restriction would improve glucose metabolism. Putative mechanisms include reduction in cellular iron levels influencing enzymatic reactions in which iron is a cofactor, to changes in inflammation, cytokine profiles or other metabolic effects [1,2]. However, it remains plausible that mild to moderate elevation of ferritin in the context of Type 2 diabetes reflects underlying inflammation. Our results suggest that in future studies, consideration should be given to assessing both iron-dependent and independent pathways by which reduction of ferritin levels could ameliorate the risk of diabetes.

Conclusions

In summary, serum ferritin levels are a robust biomarker for diabetes risk in men and women. The association of higher serum ferritin levels with diabetes is not attributable to presence of cardiometabolic risk factors or inflammatory markers such as CRP. However, the absence of parallel associations of serum iron or transferrin saturation with diabetes in the same cohort suggests that ferritin captures influences beyond iron availability which contribute to diabetes risk.

Grants and funding

BBY is recipient of a Clinical Investigator Award from the Sylvia and Charles Viertel Charitable Foundation, New South Wales, Australia. This study was funded by the National Health and Medical Research Council of Australia (Project grant 1021326). The funding sources had no involvement in the planning, analysis and writing of the manuscript.

References

1. Rajpathak, S.N., Crandall, J.P., Wylie-Rosett, J., et al. (2009) The role of iron in type 2 diabetes in humans. *Biochimica et Biopysica Acta*, **1790**, 671-681.
2. Simcox, J.A. & McClain, D.A. (2013) Iron and diabetes risk. *Cell Metabolism*, **17**, 329-341.
3. Rajpathak, S., Ma, J., Manson, J., et al. (2006) Iron intake and the risk of Type 2 diabetes in women. *Diabetes Care*, **29**, 1370-1376.
4. Jehn, M.L., Guallar, E., Clark, J.M., et al. (2007) A prospective study of plasma ferritin level and incident diabetes. *American Journal of Epidemiology*, **165**, 1047-1054.
5. Forouhi, N.G., Harding, A.H., Allison, M., et al. (2007) Elevated serum ferritin levels predict new-onset type 2 diabetes: results from the EPIC-Norfolk prospective study. *Diabetologia*, **50**, 949-956.
6. Minamiyama, Y., Takemura, S., Kodai, S., et al. (2010) Iron restriction improves type 2 diabetes in Otsuka Long-Evans Tokushima fatty rats. *American Journal of Physiology, Endocrinology and Metabolism*, **298**, E1140-E1149.
7. Fernandez-Real, J.M., Penarroja, G., Castro, A., et al. (2002) Blood letting in high-ferritin Type 2 diabetes. *Diabetes*, **51**, 1000-1004.
8. Bao, W., Rong, Y., Rong, S. & Liu, L. (2012) Dietary iron intake, body iron stores, and the risk of type 2 diabetes: a systematic review and meta-analysis. *BMC Medicine*, **10**, 119.
9. Kunutsor, S.K., Apekey, T.A., Walley, J. & Kain, K. (2013) Ferritin levels and risk of type 2 diabetes mellitus: an updated systematic review and meta-analysis of prospective evidence. *Diabetes Metabolism Research and Reviews*, **29**, 308-318.
10. Gabrielsen, J.S., Gao, Y., Simcox, J.A., et al. (2012) Adipocyte iron regulates adiponectin and insulin sensitivity. *Journal of Clinical Investigation*, **122**, 3529-3540.

11. Knuiman, M.W., Jamrozik, K., Welborn, T.A., et al. (1995) Age and secular trends in risk factors for cardiovascular disease in Busselton. *Australian Journal of Public Health*, **19**, 375-382.
12. Davis, T.M.E., Zimmett, P., Davis, W.A., et al. (2000) Autoantibodies to glutamic acid decarboxylase in diabetic patients from ta multi-ethnic Australian community: the Fremantle Diabetes Study. *Diabetic Medicine*, **17**, 667-674.
13. Holman, C.D., Bass, A.J., Rosman, D.L., et al. (2008) A decade of data linkage in Western Australia: strategic design, applications and benefits of the WA data linkage system. *Australian Health Review*, **32**, 766-777.
14. Olynyk, J.K., Cullen, D.J., Aquilia, S., et al. (1999) A population-based study of the clinical expression of the hemochromatosis gene. *New England Journal of Medicine*, **341**,718-724.
15. Hung, J., McQuillan, B.M., Thompson, P.L. & Beilby, J.P. (2008) Circulating adiponectin levels associate with inflammatory markers, insulin resistance and metabolic syndrome independent of obesity. *International Journal of Obesity*, 32, 772-779.
16. Wallace, T.M., Levy, J.C., Matthews, D.R. (2004) Use and abuse of HOMA modelling. *Diabetes Care*, **27**, 1487-1495.
17. Ford, E.S. & Cogswell, M.E. (1999) Diabetes and serum ferritin concentration among U.S. adults. *Diabetes Care*, **22**, 1978-1983.
18. Fumeron, F., Pean, F., Driss, F., et al. (2006) Ferritin and transferrin are both predictive of the onset of hyperglycemia in men and women over 3 years. *Diabetes Care*, **29**, 2090-2094.
19. Aregbesola, A., Voutilainen, S., Virtanen, J.K., et al. (2013) Body iron stores and the risk of type 2 diabetes in middle-aged men. *European Journal of Endocrinology*, **169**, 247-253.

20. Bozzini, C., Girelli, D., Olivieri, O., et al. (2005) Prevalence of body iron excess in the metabolic syndrome. *Diabetes Care*, **28**, 2061-2063.

21. Vari, I.S., Balkau, B., Kettaner, A., et al. (2007) Ferritin and transferrin are associated with metabolic syndrome abnormalities and their change over time in a general population. *Diabetes Care*, **30**, 1795-1801.

22. Salonen, J.T., Nyyssonen, K., Korpela, H., et al. (1992) High stored iron levels are associated with excess risk of myocardial infarction in Eastern Finnish men. *Circulation*, **86**, 803-811.

23. Valenti, L., Dongiovanni, P., Fargion, S. (2012) Diagnostic and therapeutic implications of the association between ferritin level and severity of non-alcoholic fatty liver disease. *World Journal of Gastroenterology*, **18**, 3782-3786.

24. Tuomainen, T-P., Nyyssonen, K., Salonen, R., et al. (1997) Body iron stores are associated with serum insulin and blood glucose concentrations. *Diabetes Care*, **20**, 426-428.

25. Jehn, M., Clark, J.M., Guallar, E. (2004) Serum ferritin and risk of metabolic syndrome in U.S. adults. *Diabetes Care*, **27**, 2422-2428.

26. Hentze, M.W., Muckenthaler, M.U., Galy, B., Camaschella, C. (2010) Two to tango: regulation of mammalian iron metabolism. *Cell*, **142**, 24-38.

27. Ellervik, C., Mandrup-Poulsen, T., Andersen, H.U., et al. (2011) Elevated transferrin saturation and risk of diabetes. *Diabetes Care*, **34**, 2256-2258.

28. Orban, E., Schwab, S., Thorand, B., Huth, C. (2013) Association of iron indices and type 2 diabetes: a meta-analysis of observational studies. *Diabetes Metabolism Research Reviews* doi: 10.1002/dmrr.2506.

29. Gabay, C., Kushner, I. (1999) Acute-phase proteins and other systemic responses to inflammation. *New England Journal of Medicine*, **340**, 448-454.

30. Muntner, P., He, J., Chen, J., et al. (2004) Prevalence of non-traditional cardiovascular risk factors among persons with impaired fasting glucose, impaired glucose tolerance, diabetes and the metabolic syndrome: analysis of the Third National Health and Nutrition Examination Survey. *Annals of Epidemiology*, **14**, 686-695.
31. Chakarova, N., Tankova, T., Atanassova, I., Dakovsska, L. (2009) Serum lipid and hsCRP levels in prediabetes – impaired fasting glucose (IFG) and impaired glucose tolerance (IGT). *Diabetes Research and Clinical Practice*, **86**, 56-60.
32. Herder, C., Carstensen, M., Ouwens, D.M. (2013) Anti-inflammatory cytokines and risk of type 2 diabetes. Diabetes, *Obesity and Metabolism*, **15** (Suppl. 3), 39-50.
33. Wrede, C.E., Buettner, R., Bollheimer, L.C., et al. (2006) Association between serum ferritin and the insulin resistance syndrome in a representative population. *European Journal of Endocrinology*, **154**, 333-340.
34. Sheu, W.H-H., Chen, Y-T., Lee, W-J., et al. (2003) A relationship between serum ferritin and the insulin resistance syndrome is present in non-diabetic women but not in non-diabetic men. *Clinical Endocrinology*, **58**, 380-385.
35. Houschyar, K.S., Ludtke, R., Dobos, G.J., et al. (2012) Effects of phlebotomy-induced reduction of body iron stores on metabolic syndrome: results from a randomised clinical trial. *BMC Medicine*, **10**, 54.

Table 1: Characteristics of the study cohort stratified according to gender and absence or presence of diabetes mellitus. P-value is for the comparison of characteristic between diabetic and non-diabetic participants within each gender.

Characteristic	Men (n=1,834)			Women (n=2,351)		
	Non-diabetic (n=1,712)	Diabetic (n=122)	p-value	Non-diabetic (n=2,210)	Diabetic (n=141)	p-value
Diabetes duration (years)		8.4 (8.7)			10.8 (11.2)	
Diabetes treatment						
Diet (%)		55.2			63.2	
Tablets (%)		44.8			33.7	
Insulin (%)		0.0			3.1	
Age (years)	49.8±17.0	65.3±11.3	<0.001	50.7±17.0	59.5±16.3	<0.001
Smoking			<0.001			0.190
Never (%)	43.0	32.0		60.6	68.1	
Ex (%)	40.5	60.7		28.3	24.1	
Current (%)	16.4	7.4		11.0	7.8	
Drinking			0.002			<0.001
Non (%)	3.9	2.5		8.0	15.6	
Ex (%)	6.4	14.8		10.7	17.0	
Light (%)	48.7	43.4		69.2	56.7	
Moderate/Heavy (%)	38.1	33.6		7.9	4.3	
Unknown (%)	2.8	5.7		4.2	6.4	
Menopause status						<0.001
Pre/OC No (%)				35.5	26.2	
Pre/OC Yes (%)				12.9	4.3	
Post/HRT No (%)				36.2	52.5	
Post/HRT Yes (%)				15.4	17.0	
CHD or stroke history (%)	7.2	23.8	<0.001	3.0	7.8	0.002
BMI (kg/m^2)	26.4 (3.4)	28.0 (3.5)	<0.001	25.5 (4.5)	28.6 (5.7)	<0.001
Waist circumference (cm)	92.5 (10.2)	100.0 (9.7)	<0.001	80.1 (11.3)	89.3 (13.5)	<0.001
SBP (mmHg)	126 (15)	138 (19)	<0.001	122 (19)	130 (21)	<0.001
DBP (mmHg)	77 (10)	80 (11)	0.003	73 (10)	74 (10)	0.090
HDL (mmol/L)	1.22 (0.31)	1.07 (0.28)	<0.001	1.55 (0.38)	1.40 (0.43)	<0.001
Triglycerides (mmol/L)	1.42 (1.04)	1.91 (1.18)	<0.001	1.16 (0.69)	1.66 (1.18)	<0.001
log Triglycerides	0.18 (0.56)	0.50 (0.53)	<0.001	0.01 (0.51)	0.31 (0.62)	<0.001
Glucose (mmol/L)	4.89 (0.54)	7.32 (2.70)	<0.001	4.73 (0.51)	6.94 (3.36)	<0.001
log Glucose (mmol/L)	1.58 (0.11)	1.94 (0.32)	<0.001	1.55 (0.11)	1.86 (0.37)	<0.001
Insulin (mU/L)	7.10 (5.01)	11.5 (7.91)	<0.001	6.71 (4.16)	14.8 (23.7)	<0.001
log Insulin (mU/L)	1.79 (0.57)	2.25 (0.62)	<0.001	1.76 (0.53)	2.28 (0.83)	<0.001
Adiponectin (mg/L)	7.75 (5.30)	7.68 (5.76)	0.887	13.3 (8.0)	11.4 (7.3)	0.007
log Adiponectin	1.84 (0.65)	1.80 (0.70)	0.426	2.41 (0.63)	2.25 (0.64)	0.003
HOMA-R	1.58 (1.23)	3.86 (3.21)	<0.001	1.44 (0.99)	4.97 (7.93)	<0.001
log HOMA-R	0.26 (0.61)	1.07 (0.75)	<0.001	0.19 (0.57)	1.02 (1.04)	<0.001
CRP (mg/L)	2.61 (6.96)	4.35 (5.37)	0.007	3.41 (9.29)	4.97 (6.69)	0.058
log CRP	0.21 (1.21)	0.91 (1.10)	<0.001	0.47 (1.23)	0.96 (1.24)	<0.001
ALT (U/L)	27.4 (16.3)	29.7 (18.6)	0.129	18.9 (14.8)	21.8 (10.5)	0.019

log ALT	3.19 (0.46)	3.25 (0.51)	0.173	2.83 (0.41)	2.99 (0.43)	<0.001
GGT (U/L)	30.2 (26.0)	42.7 (41.3)	<0.001	20.7 (16.0)	31.8 (28.7)	<0.001
log GGT	3.24 (0.52)	3.50 (0.64)	<0.001	2.89 (0.48)	3.21 (0.65)	<0.001
Ferritin (ng/mL)	208 (199)	296 (490)	<0.001	86 (96)	118 (92)	<0.001
log Ferritin	5.05 (0.80)	5.27 (0.88)	0.004	4.05 (0.95)	4.43 (0.91)	<0.001
Ferritin group*			0.017			<0.001
1 (%)	33.6	27.9		33.9	19.9	
2 (%)	33.9	27.0		33.8	28.4	
3 (%)	32.5	45.1		32.2	51.8	
Iron (µmol/l)	19.0 (5.7)	18.4 (6.3)	0.289	17.3 (6.0)	16.5 (5.1)	0.153
log Iron	2.90 (0.31)	2.86 (0.34)	0.169	2.78 (0.39)	2.75 (0.35)	0.339
Iron group*			0.204			0.081
1 (%)	35.9	41.8		33.6	31.9	
2 (%)	28.4	30.3		35.3	44.0	
3 (%)	35.6	27.9		31.1	24.1	
Transferrin saturation (%)	29.7 (10.1)	28.4 (12.2)	0.165	25.4 (13.3)	24.2 (8.5)	0.310
log Transferrin saturation	3.33 (0.35)	3.27 (0.40)	0.042	3.14 (0.44)	3.11 (0.43)	0.411
Transferrin saturation group*			0.009			0.788
1 (%)	32.4	44.3		32.7	34.8	
2 (%)	33.9	33.6		34.2	34.8	
3 (%)	33.6	22.1		33.2	30.5	

---

*	Men:	1 = Ferritin < 126	2 = 126 ≤ Ferritin < 233	3 = Ferritin ≥ 233
		1 = Iron < 17	2 = 17 ≤ Iron < 21	3 = Iron ≥ 21
		1 = TS < 25	2 = 25 ≤ TS < 33	3 = TS ≥ 33
	Women:	1 = Ferritin ≤ 41	2 = 41 < Ferritin < 92	3 = Ferritin ≥ 92
		1 = Iron < 15	2 = 15 ≤ Iron < 20	3 = Iron ≥ 20
		1 = TS < 21	2 = 21 ≤ TS < 29	3 = TS ≥ 29