Fasting serum levels of ferritin are associated with impaired pancreatic beta cell function and decreased insulin sensitivity: a population-based study

Linéa Bonfils · Christina Ellervik · Nele Friedrich · Allan Linneberg · Camilla H. Sandholt · Marit E. Jørgensen · Torben Jørgensen · Torben Hansen · Oluf Pedersen · Kristine H. Allin

Abstract

Aims/hypothesis Elevated serum ferritin levels are associated with an increased risk of type 2 diabetes, but the nature of this association remains elusive. The aim of this study was to test the hypothesis that an elevated fasting serum ferritin level is associated with an increased risk of type 2 diabetes due to its association with impaired beta cell function and decreased insulin sensitivity.

Methods We investigated 6,392 individuals from the Danish general population. Surrogate measures of beta cell function and insulin sensitivity were calculated for approximately 6,100 individuals based on OGTT examinations.

Results The ORs for type 2 diabetes were 4.2 (95% CI 2.4, 7.2) for the highest vs the lowest quintile of serum ferritin, and 17 (95% CI 8.9, 33) for serum ferritin levels ≥97.5th percentile vs <20th percentile. Elevated serum ferritin levels were associated with elevated plasma glucose levels at 0, 30 and 120 min ($p<0.001$), elevated serum insulin levels at 0 and 120 min ($p=0.02$ and $p<0.001$), decreased beta cell function estimated as the insulinogenic index and corrected insulin response ($p<0.001$), and decreased insulin sensitivity estimated by the Matsuda index of insulin sensitivity and HOMA-IR ($p<0.001$). Whereas the association with impaired beta cell function was present in both men and women, the association

---

L. Bonfils · C. H. Sandholt · T. Hansen · O. Pedersen · K. H. Allin (✉)
The Novo Nordisk Foundation Center for Basic Metabolic Research, Faculty of Health and Medical Sciences, University of Copenhagen, Universitetsparken 1, DK-2100 Copenhagen Ø, Denmark
e-mail: kristine.allin@sund.ku.dk

C. Ellervik
Department of Laboratory Medicine, Boston Children's Hospital, Boston, MA, USA

C. Ellervik · A. Linneberg
Department of Clinical Medicine, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

N. Friedrich
Institute of Clinical Chemistry and Laboratory Medicine, University Medicine Greifswald, Greifswald, Germany

A. Linneberg · T. Jørgensen
Research Centre for Prevention and Health, the Capital Region of Denmark, Copenhagen, Denmark

A. Linneberg
Department of Clinical Experimental Research, Glostrup University Hospital, Glostrup, Denmark

M. E. Jørgensen
Steno Diabetes Center, Gentofte, Denmark

T. Jørgensen
Department of Public Health, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark

T. Jørgensen
Faculty of Medicine, University of Aalborg, Aalborg, Denmark

T. Hansen
Faculty of Health Sciences, University of Southern Denmark, Odense, Denmark

O. Pedersen
Faculty of Health Sciences, University of Aarhus, Aarhus, Denmark

with decreased insulin sensitivity was observed among men and older women but not among younger women.

Conclusions/interpretation Elevated fasting serum ferritin levels are associated with surrogate measures of both impaired beta cell function and decreased insulin sensitivity. Menopause seems to modify the association with insulin sensitivity.

Keywords Corrected insulin response · Disposition index · Ferritin · HOMA-IR · Insulin · Insulin resistance · Insulinogenic index · MATSUDA · Prediabetes · Type 2 diabetes

Abbreviations

hsCRP	High-sensitivity C-reactive protein
$ISI_{Matsuda}$	Matsuda index of insulin sensitivity

Introduction

Iron is the most abundant metal in the body, and its high concentration in blood erythrocytes is essential for the utilisation of oxygen [1]. Excess iron is sequestered intracellularly in the body via the protein ferritin, and serum ferritin levels are commonly used for the clinical determination of the body's iron status [1]. The reference interval for serum ferritin is wide, comprising levels between approximately 20 and 300 μg/l among men and 15 and 200 μg/l among women. Biologically, iron is essential for the utilisation of oxygen; however, it is also a strong pro-oxidant known to catalyse the formation of reactive oxygen species, potentially causing severe damage to the insulin-producing pancreatic beta cells, muscle cells and liver cells [1–3]. Moreover, ferritin not only reflects iron stores, but is also an acute-phase reactant, commonly elevated in individuals with low-grade inflammation such as is seen in obesity, the metabolic syndrome and type 2 diabetes.

A role of iron in the pathogenesis of diabetes in humans has been suggested by several previous findings. First, iron overload in the genetic disorder hereditary haemochromatosis has been associated with an increased prevalence of diabetes [4]. In studies of rodents and up to 68 humans, iron overload due to blood transfusions or the administration of ferric nitrilotriacetic acid has been shown to induce diabetes or prediabetic conditions in the form of impaired glucose tolerance or reduced insulin sensitivity [5–10]. Second, a reduction in ferritin level, by either blood-letting or iron-chelating therapy, has been associated with improved glucose tolerance and insulin sensitivity in both healthy volunteers and patients with diabetes in studies of up to 59 participants [11–15].

Furthermore, cross-sectional studies have shown that individuals with impaired fasting glucose or type 2 diabetes exhibit higher levels of ferritin [16–18]. Several studies have

been conducted to investigate the association between serum ferritin levels and future risk of type 2 diabetes [19–27]. A recent meta-analysis of nine prospective studies including 26,339 participants (ranging from 560 participants in the smallest cohort to 7,827 in the largest) concluded that a high serum ferritin level is associated with an increased risk of incident type 2 diabetes [28]. However, it has not yet been examined in larger cohort-based studies whether this association is explained by impaired beta cell function, decreased insulin sensitivity or both. In the fasting state, elevated serum ferritin levels have been found to be associated with levels of insulin and glucose [16, 22, 29], and a few studies of up to 1,013 participants using OGTTs, IVGTTs or various clamps have shown an association between elevated ferritin levels and decreased insulin sensitivity [29–32].

The present study includes data from OGTTs on more than 6,000 individuals and therefore enables an elucidation of the glycaemic mechanisms that underlie the observed association between serum ferritin levels and risk of type 2 diabetes. Thus, the overall aim of this study was to test the hypothesis that an elevated fasting serum ferritin level is associated with an increased prevalence of type 2 diabetes due to its association with impaired beta cell function and decreased insulin sensitivity.

Methods

Study population The present study is based on the Danish population-based Inter99 study (ClinicalTrials.gov ID no. NCT00289237), which was a non-pharmacological intervention study for ischaemic heart disease [33]. A random sample of 13,016 individuals living in Copenhagen County from seven different age groups was drawn from the Civil Registration System, and a total of 6,784 (52%) attended the baseline examination, which took place from March 1999 to January 2001. Of the participants, 308 were excluded due to missing serum ferritin measurements, leaving 6,476 participants; of these, 84 participants who were receiving medical treatment for diabetes were excluded, leaving 6,392 for analysis. Written informed consent was obtained from all the participants. The study was approved by the Scientific Ethics Committee of the Capital Region of Denmark (KA 98 155) and is in accordance with the principles of the Declaration of Helsinki II.

Anthropometric and biochemical measurements Anthropometric and biochemical measures were obtained after an overnight fast. Weight (kg) and height (cm) were measured with the participants in light indoor clothes and without shoes. Plasma glucose was analysed using the hexokinase/glucose-6-phosphate dehydrogenase technique (Boehringer, Mannheim, Germany), and serum insulin (excluding des-31,32

and intact proinsulin) was measured using the AutoDELFIA insulin kit (PerkinElmer/Wallac, Turku, Finland). Serum ferritin and high-sensitivity C-reactive protein (hsCRP) were measured on serum that had been stored for approximately 13 years, using a chemiluminescence and a nephelometric assay, respectively (Siemens Healthcare Diagnostics, Eschborn, Germany). The CV was 2.6% for the ferritin assay.

Glycaemic traits Non-diabetic Inter99 participants underwent a 75 g OGTT with plasma glucose and serum insulin measured at fasting and at 30 and 120 min after the oral glucose load. The participants were characterised as having normal glucose tolerance, prediabetes (impaired fasting glucose, impaired glucose tolerance or both) or self-reported or screen-detected type 2 diabetes according to the 1999 WHO criteria [34]. Various surrogate measures of beta cell function and insulin sensitivity were calculated. Beta cell function was assessed as the insulinogenic index and corrected insulin response, which are surrogate measures of oral glucose-stimulated insulin secretion [35]. Insulin sensitivity was assessed as Matsuda index of insulin sensitivity ($ISI_{Matsuda}$) [36] and HOMA-IR. Beta cell function corrected for insulin sensitivity was expressed as the disposition index. Detailed information on the calculations of measures of beta cell function and insulin sensitivity is provided in electronic supplementary material (ESM) Table 1.

Statistical analyses Statistical analyses were performed using Stata statistical software (version 13; StataCorp, College Station, TX, USA). A *p* value <0.05 was considered statistically significant. We used a non-parametric test for trend across ordered groups to test whether serum ferritin level increased across the three groups: normal glucose tolerance, prediabetes and type 2 diabetes. To estimate the ORs of type 2 diabetes or prediabetes as a function of fasting serum ferritin levels, we used a logistic regression model that included (1) age and sex, (2) age, sex and BMI, and (3) age, sex, BMI and hsCRP levels. In addition, we used a multifactor-adjusted model including age, sex, BMI, hsCRP levels, waist circumference, hypertension (systolic BP <140 mmHg, diastolic BP <90 mmHg and no antihypertensive treatment vs systolic BP ≥140 mmHg, diastolic BP ≥90 mmHg or antihypertensive treatment), family history of diabetes (no family history, one parent with diabetes or two parents with diabetes), smoking (never, former or current smoker), alcohol use based on recommendations from the Danish Health and Medicines Authority (≤14/21 vs >14/21 units for women and men, respectively, where 1 unit = 12 g of alcohol), physical activity during leisure and commuting time (0–2 h/week, 2–4 h/week, 4–7 h/week or 7–12 h/week), LDL-cholesterol, HDL-cholesterol and triacylglycerol. Fasting serum ferritin levels were entered into the model as quintiles of ferritin, and in addition we subdivided the fifth quintile into four groups (80th–90th percentile, 90th–95th percentile, 95th–97.5th percentile and ≥97.5th percentile) to examine extremely high ferritin levels.

To test whether serum ferritin levels were associated with glycaemic traits derived from OGTTs, we used linear regression models that were adjusted for the above-mentioned covariates. To examine whether the assumptions for the linear regression analysis were being fulfilled, all the variables were inspected with regard to linearity as well as homogeneity of variance and normality of the residuals. Plasma glucose was kept in its identity form, and analyses with plasma glucose were run using robust SEs to meet assumptions concerning the normality and homogeneity of variance of the residuals. As the values of serum ferritin, serum insulin, insulinogenic index, corrected insulin response, $ISI_{Matsuda}$, HOMA-IR and disposition index displayed non-normal distributions of the residuals, these were transformed via the natural logarithm to approximate a normal distribution. Accordingly, the effect sizes are reported in per cent for a 1% change in serum ferritin. As serum ferritin levels are generally higher in men than women, all the analyses are presented for men and women both together and separately. Interaction with sex and age was tested for by introducing an interaction term between ferritin levels and sex or age in the linear regression model. The statistical software R version 3.1.0 (www.r-project.org, accessed 14 October 2014) was used to perform piecewise linear regression to examine whether there was a threshold of ferritin level that is associated with insulin resistance. A two-segment piecewise linear model adjusted for age, sex, BMI and hsCRP levels was fitted for $ISI_{Matsuda}$ and HOMA-IR, and the threshold was determined as the ferritin level at which the piecewise regression best fitted the $ISI_{Matsuda}$ and HOMA-IR, that is, where the residual SE was smallest.

Results

The baseline characteristics of the study population at the time of blood sampling are presented in Table 1. A total of 6,476 individuals were present at the health examination and had their fasting serum ferritin measured. The median ferritin level was 79 μg/l (IQR 35–152 μg/l) among all the participants, 140 μg/l (IQR 86–219 μg/l) among the men, and 42 μg/l (IQR 21–75 μg/l) among the women.

Median fasting serum ferritin levels were 68 μg/l (IQR 31–134 μg/l), 113 μg/l (IQR 53–201 μg/l) and 152 μg/l (IQR 75–271 μg/l) among the participants with normal glucose tolerance, prediabetes and type 2 diabetes, respectively (p_{trend}< 0.001). A similar pattern was observed when analysing men (p_{trend}<0.001) and women (p_{trend}<0.001) separately (data not shown). The ORs of type 2 diabetes increased in a stepwise manner with increasing fasting serum ferritin levels, and additional adjustment for BMI and hsCRP generally attenuated

Table 1 Baseline characteristics of the Inter99 study participants

Characteristic	No. of participants	All	Men	Women
Participants, n (%)	6,476	6,476	3,182 (49)	3,294 (51)
Glucose status at baseline, n (%)				
Normal glucose tolerance		4,615 (71)	2,157 (68)	2,458 (75)
Prediabetes		1,221 (19)	721 (23)	500 (15)
Impaired fasting glucose		507 (8)	372 (12)	135 (4)
Impaired glucose tolerance		507 (8)	212 (7)	295 (9)
Combined		207 (3)	137 (4)	70 (2)
Screen-detected diabetes		250 (4)	159 (5)	91 (3)
Known diabetes		132 (2)	69 (2)	63 (2)
On glucose-lowering treatment		84	48	36
Unknown glucose status		258 (4)	76 (2)	182 (6)
Anthropometric and biochemical measures[a]				
Age (years)	6,392	46 (7.9)	46 (7.9)	46 (8.0)
Serum ferritin (μg/l)	6,392	79 (35–152)	140 (86–219)	42 (21–75)
Plasma glucose (mmol/l)				
Fasting	6,388	5.5 (0.8)	5.7 (0.9)	5.4 (0.7)
30 min	6,113	8.7 (1.9)	9.2 (1.9)	8.2 (1.7)
120 min	6,125	6.2 (2.2)	6.2 (2.4)	6.3 (2.0)
Serum insulin (pmol/l)				
Fasting	6,383	34 (23–51)	36 (24–54)	32 (23–47)
30 min	6,112	242 (171–352)	248 (169–372)	237 (171– 334)
120 min	6,115	155 (94–256)	141 (78–247)	169 (112–264)
Measures of beta cell function				
Insulinogenic index	5,994	65 (40–107)	65 (40–107)	76 (48–129)
Corrected insulin response	6,096	643 (392–1,065)	559 (340–909)	738 (455–1,236)
Measures of insulin sensitivity				
$ISI_{Matsuda}$	5,990	7.8 (5.2–11.3)	7.3 (4.8–11)	8.3 (5.6–12)
HOMA-IR	6,380	1.4 (0.9–2.1)	1.5 (1.0–2.3)	1.3 (0.9–1.9)
Disposition index	5,899	538 (317–914)	473 (286–770)	620 (363–1,078)
BMI (kg/m^2)	6,389	26 (4.5)	27 (4.0)	26 (5.0)
Serum hsCRP (nmol/l)	6,063	11 (5.4–25)	9.8 (5.3–21)	11 (5.6–30)

Data are n (%), mean (SD) or median (IQR). Calculations for measures of beta cell function and insulin sensitivity as well as disposition index are described in ESM Table 1. For the insulinogenic index and corrected insulin response, 83 and 1 participants, respectively, were excluded due to negative values

[a] Participants on glucose-lowering treatment excluded

the ORs (Figs 1 and 2). The ORs adjusted for age, sex, BMI and hsCRP were 1.0 (95% CI 0.6, 1.8), 1.2 (95% CI 0.7, 2.1), 1.9 (95% CI 1.1, 3.3) and 4.2 (95% CI 2.4, 7.2) for the second, third, fourth and fifth quintiles of ferritin compared with the first quintile (p_{trend}<0.001) (Fig. 1). Stepwise increasing ORs of type 2 diabetes (p_{trend}<0.001) were also observed for the four subdivisions of the fifth quintile, with an especially high OR among participants with ferritin levels ≥97.5th vs <20th percentile (OR 17; 95% CI 8.9, 33) (Fig. 1).

As ferritin levels are highly dependent on sex, we stratified the data in terms of sex and found that increasing levels of ferritin were associated with increasing ORs for type 2

diabetes among both men and women. However, the association was strongest among men: the OR for the fifth vs the first quintile of ferritin was 6.3 (95% CI 3.4, 12) among men and 2.8 (95% CI 1.4, 5.7) among women (Fig. 2). The corresponding ORs were 31 (95% CI 13, 71) and 7.7 (95% CI 3.1, 19) for ferritin levels ≥97.5th vs <20th percentile (Fig. 2). Increasing levels of serum ferritin were also associated with a stepwise increase in OR of prediabetes among all the participants (ESM Fig. 1) and among men and women separately (ESM Fig. 2). In addition, when analysing impaired fasting glucose and impaired glucose tolerance separately, increasing levels of serum ferritin were also associated with increasing ORs of
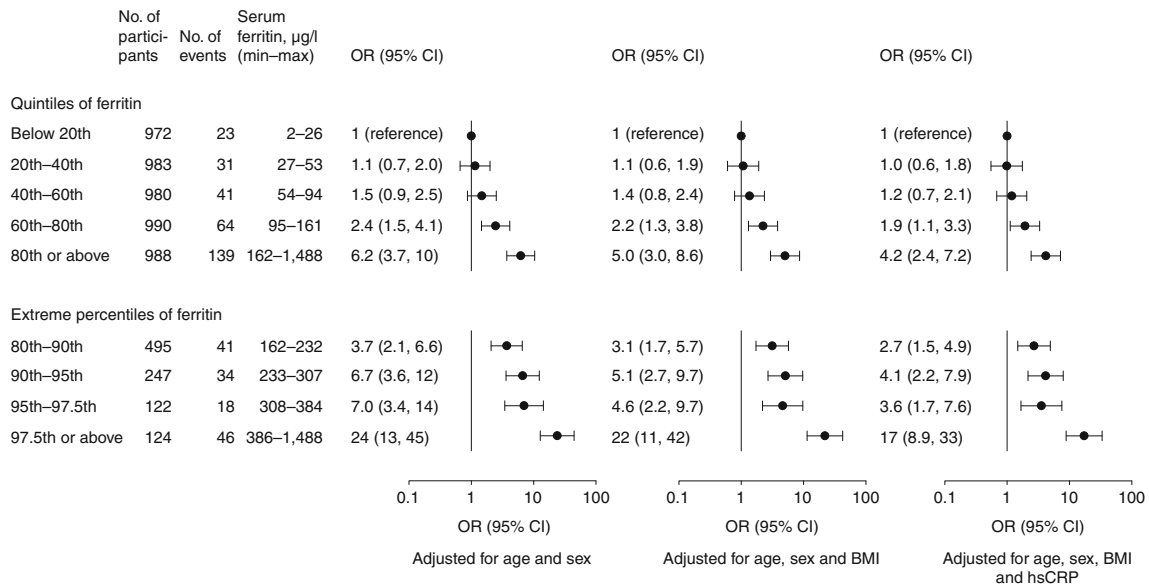
	No. of partici-pants	No. of events	Serum ferritin, µg/l (min–max)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Quintiles of ferritin						
Below 20th	972	23	2–26	1 (reference)	1 (reference)	1 (reference)
20th–40th	983	31	27–53	1.1 (0.7, 2.0)	1.1 (0.6, 1.9)	1.0 (0.6, 1.8)
40th–60th	980	41	54–94	1.5 (0.9, 2.5)	1.4 (0.8, 2.4)	1.2 (0.7, 2.1)
60th–80th	990	64	95–161	2.4 (1.5, 4.1)	2.2 (1.3, 3.8)	1.9 (1.1, 3.3)
80th or above	988	139	162–1,488	6.2 (3.7, 10)	5.0 (3.0, 8.6)	4.2 (2.4, 7.2)
Extreme percentiles of ferritin						
80th–90th	495	41	162–232	3.7 (2.1, 6.6)	3.1 (1.7, 5.7)	2.7 (1.5, 4.9)
90th–95th	247	34	233–307	6.7 (3.6, 12)	5.1 (2.7, 9.7)	4.1 (2.2, 7.9)
95th–97.5th	122	18	308–384	7.0 (3.4, 14)	4.6 (2.2, 9.7)	3.6 (1.7, 7.6)
97.5th or above	124	46	386–1,488	24 (13, 45)	22 (11, 42)	17 (8.9, 33)
				OR (95% CI) Adjusted for age and sex	OR (95% CI) Adjusted for age, sex and BMI	OR (95% CI) Adjusted for age, sex, BMI and hsCRP

Fig. 1 ORs of type 2 diabetes according to fasting serum ferritin levels. The numbers of participants and events are provided for the age- and sex-adjusted analysis. There was no information on BMI for one participant and on serum hsCRP for 289 participants. $p_{trend} < 0.001$ for all analyses

both outcomes, and when impaired fasting glucose was defined as a fasting plasma glucose level ≥5.6 mmol/l, the results for prediabetes as well as for impaired fasting glucose were similar to the main results (data not shown).

After adjustment for age, sex, BMI and hsCRP, elevated fasting serum ferritin levels were associated with elevated plasma glucose levels at fasting, 30 min and 120 min ($p < 0.001$), elevated serum insulin levels at fasting and 120 min ($p = 0.02$ and $p < 0.001$), decreased beta cell function
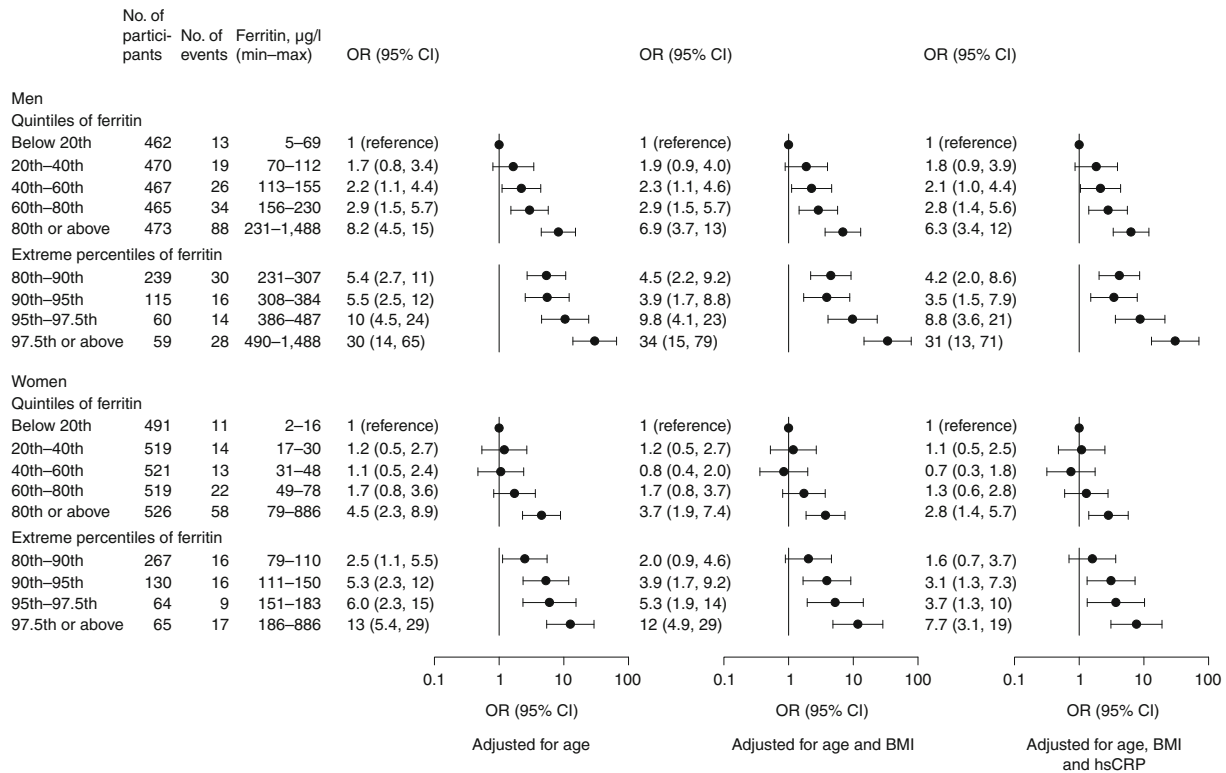
	No. of partici-pants	No. of events	Ferritin, µg/l (min–max)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Men						
Quintiles of ferritin						
Below 20th	462	13	5–69	1 (reference)	1 (reference)	1 (reference)
20th–40th	470	19	70–112	1.7 (0.8, 3.4)	1.9 (0.9, 4.0)	1.8 (0.9, 3.9)
40th–60th	467	26	113–155	2.2 (1.1, 4.4)	2.3 (1.1, 4.6)	2.1 (1.0, 4.4)
60th–80th	465	34	156–230	2.9 (1.5, 5.7)	2.9 (1.5, 5.7)	2.8 (1.4, 5.6)
80th or above	473	88	231–1,488	8.2 (4.5, 15)	6.9 (3.7, 13)	6.3 (3.4, 12)
Extreme percentiles of ferritin						
80th–90th	239	30	231–307	5.4 (2.7, 11)	4.5 (2.2, 9.2)	4.2 (2.0, 8.6)
90th–95th	115	16	308–384	5.5 (2.5, 12)	3.9 (1.7, 8.8)	3.5 (1.5, 7.9)
95th–97.5th	60	14	386–487	10 (4.5, 24)	9.8 (4.1, 23)	8.8 (3.6, 21)
97.5th or above	59	28	490–1,488	30 (14, 65)	34 (15, 79)	31 (13, 71)
Women						
Quintiles of ferritin						
Below 20th	491	11	2–16	1 (reference)	1 (reference)	1 (reference)
20th–40th	519	14	17–30	1.2 (0.5, 2.7)	1.2 (0.5, 2.7)	1.1 (0.5, 2.5)
40th–60th	521	13	31–48	1.1 (0.5, 2.4)	0.8 (0.4, 2.0)	0.7 (0.3, 1.8)
60th–80th	519	22	49–78	1.7 (0.8, 3.6)	1.7 (0.8, 3.7)	1.3 (0.6, 2.8)
80th or above	526	58	79–886	4.5 (2.3, 8.9)	3.7 (1.9, 7.4)	2.8 (1.4, 5.7)
Extreme percentiles of ferritin						
80th–90th	267	16	79–110	2.5 (1.1, 5.5)	2.0 (0.9, 4.6)	1.6 (0.7, 3.7)
90th–95th	130	16	111–150	5.3 (2.3, 12)	3.9 (1.7, 9.2)	3.1 (1.3, 7.3)
95th–97.5th	64	9	151–183	6.0 (2.3, 15)	5.3 (1.9, 14)	3.7 (1.3, 10)
97.5th or above	65	17	186–886	13 (5.4, 29)	12 (4.9, 29)	7.7 (3.1, 19)
				OR (95% CI) Adjusted for age	OR (95% CI) Adjusted for age and BMI	OR (95% CI) Adjusted for age, BMI and hsCRP

Fig. 2 ORs of type 2 diabetes according to fasting serum ferritin levels stratified by sex. The numbers of participants and events are provided for the age-adjusted analysis. There was no information on BMI for one woman, and on serum hsCRP for 122 men and 167 women. $p_{trend} < 0.001$ for all analyses

assessed as the insulinogenic index and corrected insulin response ($p<0.001$), and decreased insulin sensitivity assessed as the $ISI_{Matsuda}$ and HOMA-IR ($p<0.001$) (Table 2). Generally, adjustment for BMI and hsCRP resulted in attenuated effect sizes compared with the effect sizes from the age- and sex-adjusted analyses (Table 2).

Interaction analyses suggested that sex modified the association between ferritin levels and 120 min plasma glucose, fasting serum insulin, 120 min serum insulin, $ISI_{Matsuda}$ and HOMA-IR ($p_{int}<0.001$) (Table 3). Serum ferritin levels were only associated with fasting insulin, $ISI_{Matsuda}$ and HOMA-IR among men but not among women. Furthermore, serum ferritin levels were more strongly associated with 120 min plasma glucose and 120 min serum insulin among men than women (Table 3). To examine whether the discrepancies in the association between ferritin levels and measures of insulin sensitivity could be explained by women having menstrual periods and thereby losing iron, or by sex hormone differences, we stratified women by age below or above 52 years, which is the median age of menopause among Danish women [37]. Women <52 years had a median ferritin level of 35 μg/l (IQR 18–62 μg/l) whereas women ≥52 years had a median ferritin level of 72 μg/l (IQR 45–116 μg/l).

Generally, when stratifying by age, the effect sizes among younger women resembled the effect sizes among all women, whereas the effect sizes among older women resembled the effect sizes observed among men (Table 4). Significant interactions between serum ferritin levels and age <52 or ≥52 years were observed for fasting insulin ($p_{int}=0.003$), 120 min insulin ($p_{int}=0.002$), $ISI_{Matsuda}$ ($p_{int}=0.006$) and HOMA-IR ($p_{int}=0.02$). Thus, elevated serum ferritin levels were associated with a decreased fasting serum insulin level among younger women but increased fasting serum insulin among older women, whereas elevated serum ferritin levels were associated with 120 min serum insulin, $ISI_{Matsuda}$ and HOMA-IR among older women but not among younger women (Table 4). When stratifying by age 52 years among men, we found no major differences between the younger and older group of men, and no significant interaction between ferritin levels and age <52 or ≥52 years (data not shown).

By using a piecewise linear regression model, we found a negative association between ferritin levels and insulin resistance among individuals with serum ferritin levels ≤52 μg/l ($ISI_{Matsuda}$ [$n=2,010$] 0.04; 95% CI 0.005, 0.07; HOMA-IR [$n=2,158$] −0.04; 95% CI −0.08, −0.01) but a positive association among individuals with levels >52 μg/l ($ISI_{Matsuda}$ [$n=3,666$] −0.13; 95% CI −0.16, −0.10; HOMA-IR [$n=3,891$] 0.11; 95% CI 0.08, 0.14). However, when individuals with ferritin levels <15 μg/l (the lower limit of the reference interval for women) were excluded ($n=473$ for $ISI_{Matsuda}$ and $n=518$ for HOMA-IR), the negative association

Table 2 Associations between fasting serum ferritin levels and glycaemic traits obtained during OGTTs in the Inter99 cohort

Variable	Adjusted for age and sex			Adjusted for age, sex and BMI			Adjusted for age, sex, BMI and hsCRP		
	n	Effect (95% CI)	p value	n	Effect (95% CI)	p value	n	Effect (95% CI)	p value
Plasma glucose (mmol/l)									
Fasting	6,388	0.11 (0.08, 0.13)	<0.001	6,385	0.09 (0.06, 0.11)	<0.001	6,056	0.08 (0.06, 0.11)	<0.001
30 min	6,113	0.24 (0.19, 0.29)	<0.001	6,112	0.19 (0.14, 0.24)	<0.001	5,794	0.16 (0.11, 0.21)	<0.001
120 min	6,125	0.46 (0.39, 0.53)	<0.001	6,124	0.40 (0.33, 0.46)	<0.001	5,806	0.36 (0.29, 0.43)	<0.001
Serum insulin (%)									
Fasting	6,383	0.06 (0.04, 0.07)	<0.001	6,380	0.02 (0.01, 0.04)	0.002	6,052	0.02 (0.003, 0.03)	0.02
30 min	6,112	0.008 (−0.009, 0.03)	0.35	6,111	−0.02 (−0.03, −0.00)	0.05	5,792	−0.02 (−0.04, −0.001)	0.04
120 min	6,115	0.15 (0.12, 0.17)	<0.001	6,114	0.11 (0.09, 0.13)	<0.001	5,798	0.10 (0.07, 0.12)	<0.001
Measures of beta cell function (%)									
Insulinogenic index	5,994	−0.04 (−0.07, −0.02)	<0.001	5,993	−0.06 (−0.08, −0.03)	<0.001	5,684	−0.05 (−0.07, −0.03)	<0.001
Corrected insulin response	6,096	−0.07 (−0.09, −0.05)	<0.001	6,095	−0.08 (−0.10, −0.06)	<0.001	5,777	−0.07 (−0.09, −0.05)	<0.001
Measures of insulin sensitivity (%)									
$ISI_{Matsuda}$	5,990	−0.09 (−0.10, −0.07)	<0.001	5,989	−0.05 (−0.06, −0.04)	<0.001	5,676	−0.04 (−0.06, −0.03)	<0.001
HOMA-IR	6,380	0.07 (0.06, 0.09)	<0.001	6,377	0.04 (0.02, 0.05)	<0.001	6,049	0.03 (0.02, 0.05)	<0.001
Disposition index (%)	5,899	−0.13 (−0.15, −0.10)	<0.001	5,898	−0.11 (−0.13, −0.08)	<0.001	5,593	−0.09 (−0.11, −0.07)	<0.001

All traits except for plasma glucose were logarithmically transformed before analysis. Effects (95% CI) for plasma glucose are given for a 1 unit increase in $\log_e$(ferritin), whereas effects on serum insulin, measures of beta cell function, measures of insulin sensitivity and disposition index are given for a 1% increase in ferritin. Calculations of measures of beta cell function, insulin sensitivity and disposition index were carried out as described in ESM Table 1

Table 3 Associations between fasting serum ferritin levels and glycaemic traits obtained during OGTTs in the Inter99 cohort stratified by sex

Variable	Men			Women			
	n	Effect (95% CI)	p value	n	Effect (95% CI)	p value	$p_{interaction}$
Plasma glucose (mmol/l)							
Fasting	2,990	0.10 (0.06, 0.14)	<0.001	3,066	0.08 (0.04, 0.11)	<0.001	0.12
30 min	2,925	0.17 (0.08, 0.25)	<0.001	2,869	0.15 (0.08, 0.21)	<0.001	0.84
120 min	2,915	0.52 (0.40, 0.63)	<0.001	2,891	0.27 (0.18, 0.35)	<0.001	<0.001
Serum insulin (%)							
Fasting	2,990	0.05 (0.03, 0.08)	<0.001	3,062	−0.01 (−0.03, 0.01)	0.34	<0.001
30 min	2,923	−0.009 (−0.04, 0.02)	0.53	2,869	−0.03 (−0.06, −0.01)	0.001	0.04
120 min	2,911	0.17 (0.14, 0.21)	<0.001	2,887	0.04 (0.009, 0.06)	0.008	<0.001
Measures of beta cell function (%)							
Insulinogenic index	2,887	−0.04 (−0.07, −0.006)	0.02	2,797	−0.06 (−0.09, −0.03)	<0.001	0.06
Corrected insulin response	2,919	−0.06 (−0.09, −0.02)	0.001	2,858	−0.08 (−0.11, −0.05)	<0.001	0.05
Measures of insulin sensitivity (%)							
ISI$_{Matsuda}$	2,875	−0.08 (−0.10, −0.06)	<0.001	2,801	−0.008 (−0.03, 0.01)	0.43	<0.001
HOMA-IR	2,988	0.07 (0.04, 0.09)	<0.001	3,061	0.002 (−0.02, 0.02)	0.89	<0.001
Disposition index (%)	2,847	−0.12 (−0.15, −0.08)	<0.001	2,746	−0.07 (−0.10, −0.03)	<0.001	0.09

All traits except for plasma glucose were logarithmically transformed before analysis. Effects (95% CI) for plasma glucose are given for a 1 unit increase in log$_e$(ferritin), whereas effects on serum insulin, measures of beta cell function, measures of insulin sensitivity and disposition index are given for a 1% increase in ferritin level. Analyses are adjusted for age, BMI and hsCRP. Calculations of measures of beta cell function, insulin sensitivity and disposition index were carried out as described in ESM Table 1

with insulin resistance observed among individuals with levels ≤52 μg/l was insignificant ($p=0.46$ and 0.90 for ISI$_{Matsuda}$ and HOMA-IR, respectively).

Elevated levels of triacylglycerol are known to be associated with decreased insulin sensitivity, and in this study increasing levels of triacylglycerol were associated with

Table 4 Associations between fasting serum ferritin levels and glycaemic traits obtained during OGTTs in women from the Inter99 cohort stratified by age

Variable		Women <52 years Effect (95% CI)	p value		Women ≥52 years Effect (95% CI)	p value	$p_{interaction}$
	n			n			
Plasma glucose (mmol/l)							
Fasting	2,368	0.07 (0.03, 0.11)	0.001	698	0.15 (0.07, 0.22)	<0.001	0.17
30 min	2,205	0.13 (0.05, 0.20)	0.001	664	0.26 (0.09, 0.43)	0.003	0.63
120 min	2,227	0.21 (0.12, 0.31)	<0.001	664	0.48 (0.26, 0.70)	<0.001	0.24
Serum insulin (%)							
Fasting	2,367	−0.03 (−0.05, −0.005)	0.02	695	0.05 (−0.0007, 0.09)	0.05	0.003
30 min	2,202	−0.04 (−0.06, −0.01)	0.002	667	−0.03 (−0.08, 0.02)	0.26	0.51
120 min	2,222	0.01 (−0.02, 0.04)	0.40	665	0.10 (0.04, 0.17)	0.002	0.002
Measures of beta cell function (%)							
Insulinogenic index	2,144	−0.06 (−0.09, −0.02)	0.002	653	−0.06 (−0.13, 0.005)	0.07	0.77
Corrected insulin response	2,196	−0.08 (−0.11, −0.02)	<0.001	662	−0.11 (−0.18, −0.04)	0.002	0.22
Measures of insulin sensitivity (%)							
ISI$_{Matsuda}$	2,155	0.01 (−0.01, 0.03)	0.31	646	−0.07 (−0.12, −0.02)	0.003	0.006
HOMA-IR	2,366	−0.02 (−0.04, 0.006)	0.14	695	0.07 (0.02, 0.12)	0.007	0.02
Disposition index (%)	2,107	−0.05 (−0.09, −0.008)	0.02	644	−0.13 (−0.20, −0.05)	0.001	0.19

All traits except for plasma glucose were logarithmically transformed before analysis. Effects (95% CI) for plasma glucose are given for a 1 unit increase in log$_e$(ferritin), whereas the effects on serum insulin, measures of beta cell function, measures of insulin sensitivity and disposition index are given for a 1% increase in ferritin level. Analyses are adjusted for age, BMI and hsCRP. Calculations of measures of beta cell function, insulin sensitivity and disposition index were carried out as described in ESM Table 1

elevated levels of serum ferritin, a decreased $ISI_{Matsuda}$ and an elevated HOMA-IR. However, we found no statistically significant interaction between serum ferritin and serum triacylglycerol on either the $ISI_{Matsuda}$ ($p=0.09$) or HOMA-IR ($p=0.10$).

Sensitivity analyses Additional adjustment for waist circumference, hypertension, family history of diabetes, smoking, alcohol use, physical activity, LDL-cholesterol, HDL-cholesterol and triacylglycerol resulted in an OR of type 2 diabetes for the fifth vs the first quintile of ferritin of 3.3 (95% CI 1.7, 6.5) and decreased the effect sizes for surrogate measures of beta cell function and insulin sensitivity (ESM Fig. 3 and ESM Table 2). Notably, the effect sizes for the associations between elevated serum ferritin levels and $ISI_{Matsuda}$ and HOMA-IR were directionally consistent with the results from models adjusted for age, sex, BMI and hsCRP only, although they were statistically insignificant. Importantly, however, 830 participants were excluded from the fully adjusted model due to lipid-lowering treatment ($n=78$) or missing information on one or more covariates ($n=752$), resulting in reduced statistical power.

As serum ferritin levels are influenced by a variety of diseases or conditions, for example haematological disorders and acute inflammatory conditions, we analysed the data after excluding individuals with very high serum ferritin levels, that is, ≥97.5th percentile. Generally, the effect sizes decreased slightly, but elevated serum ferritin levels remained associated with elevated plasma glucose and surrogate measures of decreased beta cell function and insulin sensitivity (ESM Table 3). Likewise, after the exclusion of individuals with serum ferritin levels ≥97.5th percentile, the OR of type 2 diabetes for the fifth vs the first quintile of ferritin decreased to 3.0 (95% CI 1.7, 5.3) (ESM Fig. 4).

Discussion

We evaluated ferritin status and glycaemic traits obtained from OGTTs in more than 6,000 Danish individuals and observed that elevated fasting serum ferritin levels were associated with surrogate measures of impaired beta cell function and decreased insulin sensitivity. Whereas the association with impaired beta cell function was present in both men and women, the association with decreased insulin sensitivity was observed among men and older women but not among younger women. We also replicated previous findings of an association between elevated ferritin levels and increased odds of type 2 diabetes. Of interest, our examination of extremely high levels of serum ferritin showed stepwise increasing odds of type 2 diabetes, with a particularly high risk among people with very high fasting serum ferritin levels.

The precise mechanisms underlying the crosstalk between iron stores reflected in serum ferritin levels and the development of type 2 diabetes remain unclarified. Three alternative interpretations of our findings may exist.

First, elevated ferritin levels may, in concert with other pathogenic factors, play a causal role in the development of impaired beta cell function and decreased insulin sensitivity. Iron mediates oxidative stress [38], and oxidative stress has many deleterious effects [1–3]. Studies in mouse models of the genetic disorder haemochromatosis have shown that pancreatic beta cells have an extreme susceptibility to oxidative damage that translates into increased apoptosis and a desensitisation of glucose-induced insulin secretion, which together result in decreased insulin secretory capacity [39]. Moreover, oxidative stress has been shown to result in impaired insulin endocytosis in endothelial cell lines [40], and iron overload has been shown to result in disturbed metabolic activity of the liver and skeletal muscle [39, 41] together supporting a role of ferritin in insulin resistance.

Second, our findings may represent reverse causality; that is, impaired glucose regulation may lead to elevated levels of ferritin rather than the converse. In support of this hypothesis, cell studies have shown that insulin regulates the transcription of ferritin and stimulates iron uptake in fat cells [42, 43].

Third, other factors, such as low-grade inflammation, may elevate both circulating ferritin levels and the risk of type 2 diabetes, a hypothesis that is supported by the fact that serum ferritin is an acute-phase reactant that is elevated in, for example, the metabolic syndrome. In the present study, additional adjustment for hsCRP generally resulted in only slightly attenuated effect sizes, suggesting that the association between elevated serum ferritin levels and impaired glucose regulation did not simply reflect confounding by low-grade inflammation.

We confirm the findings of previous studies showing a positive association between serum ferritin levels and fasting plasma glucose and serum insulin levels [16, 18], and of previous smaller studies showing an association with 120 min plasma glucose and 120 min insulin levels [29, 31]. However, our observation of an association between elevated ferritin levels and impaired beta cell function is in contrast to a study of 538 non-diabetic persons undergoing an OGTT and 69 non-diabetic persons undergoing a hyperglycaemic clamp in which no associations were observed between ferritin levels and beta cell function [31]. Our finding of an association with decreased insulin sensitivity assessed as the $ISI_{Matsuda}$ and HOMA-IR is in accordance with the findings of previous smaller studies [30, 31]. A study of 257 non-diabetic people applied euglycaemic–hyperinsulinaemic clamps—the gold standard to assess insulin sensitivity—and found that elevated serum ferritin levels were associated with decreased insulin sensitivity [31]. These results were supported by a study of

138 men that used a frequently sampled IVGTT to evaluate the association between ferritin and insulin sensitivity [30].

Our observation that elevated serum ferritin levels are associated with decreased insulin sensitivity in men and older women but not in younger women is intriguing. In agreement with our findings, a study of approximately 12,000 Koreans reported that serum ferritin was associated with HOMA-IR in men but not in women (pre- and postmenopausal) [44], whereas another study of 6,311 Koreans found an association between serum ferritin and HOMA-IR in men and postmenopausal women but not in premenopausal women [45]. In contrast, a significant association between serum ferritin and HOMA-IR has been reported in Chinese women (pre- and postmenopausal) ($n=277$) but not in men ($n=140$) [32].

Our results from a stratified analysis based on the median age of menopause suggest that the difference in effect sizes seen between men and women may rely on differences between pre- and postmenopausal women. Thus, the association between insulin sensitivity and serum ferritin seen in older but not younger women could simply signify that younger women have physiologically lower levels of ferritin, not associated with impaired glucose regulation. In support of this notion, our results suggest that there may be a threshold of serum ferritin of 52 μg/l above which elevated serum ferritin levels are associated with surrogate measures of insulin resistance. At menopause, the production of oestrogen declines and menstrual blood loss ceases, resulting in a rise in ferritin levels. The reduction in oestrogen levels seen at menopause has also been shown to increase levels of oxidative stress [46], probably reinforcing the association between ferritin and insulin sensitivity. The fact that our analysis of men stratified by age showed no difference between age groups supports an interaction between ferritin levels and menopausal status on insulin sensitivity, and not simply an interaction with age.

This study is, to our knowledge, the largest to date to examine the association between serum ferritin levels and glycaemic traits through OGTT data. Nevertheless, some limitations need to be addressed. Although ferritin is a widely used marker of iron status, the physiological role of serum ferritin and its relation to the intracellular labile iron pool has not been fully clarified, and additional analyses of other biomarkers of iron status are needed to understand in depth the role of iron in the pathogenesis of type 2 diabetes [47]. Moreover, a full blood count profile including haemoglobin was not measured in this study, precluding an analysis of the interactions between anaemia and other factors in terms of a full blood count profile and ferritin levels. In addition, the exclusion of individuals based on information on diseases and conditions influencing ferritin levels (e.g. inflammatory conditions, haemochromatosis, transfusions and iron therapy) would have been preferable to our simplistic exclusion of individuals with very high ferritin levels. Although the results from our study suggest that confounding by low-grade inflammation cannot explain the observed association between elevated serum ferritin levels and impaired glucose regulation, it is important to underline that ferritin is also an inflammatory biomarker that is elevated in the metabolic syndrome.

Along these lines, the causality of the observed association between serum ferritin and type 2 diabetes cannot be determined from this cross-sectional study. Mendelian randomisation studies could help to inform us whether serum ferritin is likely to play a causal role in development of type 2 diabetes and consequently whether the initiation of intervention studies would be reasonable. One may speculate that a Westernised diet contributes to the risk of diabetes not only through an excess caloric intake, but also through an excess iron content in the diet and via the use of iron supplements. If Mendelian randomisation studies and interventional studies confirm an aetiological role of iron in the pathogenesis of type 2 diabetes, a reduced dietary iron intake, especially in men and postmenopausal women with additional risk factors for type 2 diabetes, could be a logical consequence.

In conclusion, we found that the observed association between elevated fasting serum ferritin levels and increased risk of type 2 diabetes is explained by an association with both surrogate measures of impaired beta cell function and surrogate measures of decreased insulin sensitivity. Interestingly, menopause seems to modify the association with insulin sensitivity since the association with decreased insulin sensitivity was only observed among men and older women but not among younger women.

interpreted the data and wrote the first draft of the manuscript. All authors contributed to the interpretation of the data, critically reviewed and edited the manuscript, and approved the version to be published.

References

1. Puntarulo S (2005) Iron, oxidative stress and human health. Mol Asp Med 26:299–312
2. Hansen JB, Moen IW, Mandrup-Poulsen T (2014) Iron: the hard player in diabetes pathophysiology. Acta Physiol (Oxf) 210:717–732
3. Rajpathak SN, Crandall JP, Wylie-Rosett J, Kabat GC, Rohan TE, Hu FB (2009) The role of iron in type 2 diabetes in humans. Biochim Biophys Acta 1790:671–681
4. McClain DA, Abraham D, Rogers J et al (2006) High prevalence of abnormal glucose homeostasis secondary to decreased insulin secretion in individuals with hereditary haemochromatosis. Diabetologia 49:1661–1669
5. Awai M, Narasaki M, Yamanoi Y, Seno S (1979) Induction of diabetes in animals by parenteral administration of ferric nitrilotriacetate. A model of experimental hemochromatosis. Am J Pathol 95:663–673
6. Hatunic M, Finucane FM, Brennan AM, Norris S, Pacini G, Nolan JJ (2010) Effect of iron overload on glucose metabolism in patients with hereditary hemochromatosis. Metabolism 59:380–384
7. Dmochowski K, Finegood DT, Francombe W, Tyler B, Zinman B (1993) Factors determining glucose tolerance in patients with thalassemia major. J Clin Endocrinol Metab 77:478–483
8. Cario H, Holl RW, Debatin KM, Kohne E (2003) Insulin sensitivity and beta-cell secretion in thalassaemia major with secondary haemochromatosis: assessment by oral glucose tolerance test. Eur J Pediatr 162:139–146
9. Merkel PA, Simonson DC, Amiel SA et al (1988) Insulin resistance and hyperinsulinemia in patients with thalassemia major treated by hypertransfusion. N Engl J Med 318:809–814
10. Schafer AI, Cheron RG, Dluhy R et al (1981) Clinical consequences of acquired transfusional iron overload in adults. N Engl J Med 304:319–324
11. Brittenham GM, Griffith PM, Nienhuis AW et al (1994) Efficacy of deferoxamine in preventing complications of iron overload in patients with thalassemia major. N Engl J Med 331:567–573
12. Facchini FS (1998) Effect of phlebotomy on plasma glucose and insulin concentrations. Diabetes Care 21:2190
13. Fernandez-Real JM, Penarroja G, Castro A, Garcia-Bragado F, Hernandez-Aguado I, Ricart W (2002) Blood letting in high-ferritin type 2 diabetes: effects on insulin sensitivity and beta-cell function. Diabetes 51:1000–1004
14. Hatunic M, Finucane FM, Norris S, Pacini G, Nolan JJ (2010) Glucose metabolism after normalization of markers of iron overload by venesection in subjects with hereditary hemochromatosis. Metabolism 59:1811–1815
15. Hua NW, Stoohs RA, Facchini FS (2001) Low iron status and enhanced insulin sensitivity in lacto-ovo vegetarians. Br J Nutr 86:515–519
16. Ford ES, Cogswell ME (1999) Diabetes and serum ferritin concentration among U.S. adults. Diabetes Care 22:1978–1983
17. Hernandez C, Lecube A, Carrera A, Simo R (2005) Soluble transferrin receptors and ferritin in Type 2 diabetic patients. Diabet Med 22:97–101
18. Van CA, van Campenhout C, Lagrou AR et al (2006) Impact of diabetes mellitus on the relationships between iron-, inflammatory- and oxidative stress status. Diabetes Metab Res Rev 22:444–454
19. Aregbesola A, Voutilainen S, Virtanen JK, Mursu J, Tuomainen TP (2013) Body iron stores and the risk of type 2 diabetes in middle-aged men. Eur J Endocrinol 169:247–253
20. Forouhi NG, Harding AH, 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
21. 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: the data from an epidemiological study on the Insulin Resistance Syndrome (DESIR) study. Diabetes Care 29:2090–2094
22. Jehn ML, Guallar E, Clark JM et al (2007) A prospective study of plasma ferritin level and incident diabetes: the Atherosclerosis Risk in Communities (ARIC) Study. Am J Epidemiol 165:1047–1054
23. Jiang R, Manson JE, Meigs JB, Ma J, Rifai N, Hu FB (2004) Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. JAMA 291:711–717
24. Le TD, Bae S, Ed HC, Singh KP, Blair SN, Shang N (2008) Effects of cardiorespiratory fitness on serum ferritin concentration and incidence of type 2 diabetes: evidence from the Aerobics Center Longitudinal Study (ACLS). Rev Diabet Stud 5:245–252
25. Montonen J, Boeing H, Steffen A et al (2012) Body iron stores and risk of type 2 diabetes: results from the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study. Diabetologia 55:2613–2621
26. Rajpathak SN, Wylie-Rosett J, Gunter MJ et al (2009) Biomarkers of body iron stores and risk of developing type 2 diabetes. Diabetes Obes Metab 11:472–479
27. Salomaa V, Havulinna A, Saarela O et al (2010) Thirty-one novel biomarkers as predictors for clinically incident diabetes. PLoS One 5:e10100
28. Kunutsor SK, Apekey TA, 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 Metab Res Rev 29:308–318
29. Tuomainen TP, Nyyssonen K, Salonen R et al (1997) Body iron stores are associated with serum insulin and blood glucose concentrations. Population study in 1,013 eastern Finnish men. Diabetes Care 20:426–428
30. Fernandez-Real JM, Moreno JM, Chico B, Lopez-Bermejo A, Ricart W (2007) Circulating visfatin is associated with parameters of iron metabolism in subjects with altered glucose tolerance. Diabetes Care 30:616–621
31. Haap M, Fritsche A, Mensing HJ, Haring HU, Stumvoll M (2003) Association of high serum ferritin concentration with glucose intolerance and insulin resistance in healthy people. Ann Intern Med 139:869–871
32. Sheu WH, Chen YT, Lee WJ, Wang CW, Lin LY (2003) A relationship between serum ferritin and the insulin resistance syndrome is present in non-diabetic women but not in non-diabetic men. Clin Endocrinol (Oxf) 58:380–385
33. Jorgensen T, Borch-Johnsen K, Thomsen TF, Ibsen H, Glumer C, Pisinger C (2003) A randomized non-pharmacological intervention study for prevention of ischaemic heart disease: baseline results Inter99. Eur J Cardiovasc Prev Rehabil 10:377–386
34. World Health Organization (1999) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus. WHO, Geneva
35. Hanson RL, Pratley RE, Bogardus C et al (2000) Evaluation of simple indices of insulin sensitivity and insulin secretion for use in epidemiologic studies. Am J Epidemiol 151:190–198
36. Matsuda M, DeFronzo RA (1999) Insulin sensitivity indices obtained from oral glucose tolerance testing: comparison with the euglycemic insulin clamp. Diabetes Care 22:1462–1470
37. The Danish e-Health Portal (2014) Klimakteriet. Available from www.sundhed.dk/sundhedsfaglig/laegehaandbogen/gynaekologi/tilstande-og-sygdomme/diverse/klimakteriet, Accessed 1 Aug 2014

38. Broedbaek K, Poulsen HE, Weimann A et al (2009) Urinary excretion of biomarkers of oxidatively damaged DNA and RNA in hereditary hemochromatosis. Free Radic Biol Med 47:1230–1233

39. Cooksey RC, Jouihan HA, Ajioka RS et al (2004) Oxidative stress, beta-cell apoptosis, and decreased insulin secretory capacity in mouse models of hemochromatosis. Endocrinology 145:5305–5312

40. Bertelsen M, Anggard EE, Carrier MJ (2001) Oxidative stress impairs insulin internalization in endothelial cells in vitro. Diabetologia 44:605–613

41. Huang J, Jones D, Luo B et al (2011) Iron overload and diabetes risk: a shift from glucose to fatty acid oxidation and increased hepatic glucose production in a mouse model of hereditary hemochromatosis. Diabetes 60:80–87

42. Davis RJ, Corvera S, Czech MP (1986) Insulin stimulates cellular iron uptake and causes the redistribution of intracellular transferrin receptors to the plasma membrane. J Biol Chem 261:8708–8711

43. Yokomori N, Iwasa Y, Aida K, Inoue M, Tawata M, Onaya T (1991) Transcriptional regulation of ferritin messenger ribonucleic acid levels by insulin in cultured rat glioma cells. Endocrinology 128: 1474–1480

44. Kim CH, Kim HK, Bae SJ, Park JY, Lee KU (2011) Association of elevated serum ferritin concentration with insulin resistance and impaired glucose metabolism in Korean men and women. Metabolism 60:414–420

45. Lee BK, Kim Y, Kim YI (2011) Association of serum ferritin with metabolic syndrome and diabetes mellitus in the South Korean general population according to the Korean national health and nutrition examination survey 2008. Metabolism 60:1416–1424

46. Doshi SB, Agarwal A (2013) The role of oxidative stress in menopause. J Midlife Health 4:140–146

47. Adams PC (2012) Diabetes: serum ferritin levels and T2DM—are body iron stores elevated? Nat Rev Endocrinol 8:573–575