Biomarkers of iron metabolism are independently associated with impaired glucose metabolism and type 2 diabetes: the KORA F4 study

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Abstract

Objective: Iron has been suggested to play a role in the etiology of type 2 diabetes mellitus (T2DM). Except for ferritin, evidence is sparse for other markers of iron metabolism that are regulated differently and might act through independent pathways. We therefore investigated the associations of serum ferritin, transferrin, soluble transferrin receptor (sTfR), transferrin saturation (TSAT), sTfR-to-log$_{10}$ferritin (sTfR-F) index, and iron with impaired glucose metabolism (IGM/'prediabetes'), T2DM, and four continuous glucose and insulin traits.
Design and methods: Data from 2893 participants of the population-based Cooperative Health Research in the Region of Augsburg (KORA) F4 study (Germany) was investigated through regression analysis. The results were adjusted for socio-demographic, life-style, and obesity measures as well as metabolic, inflammatory, and other iron biomarkers following a step-wise approach. Non-linearity was tested by adding a non-linear spline component to the model.
Results: Ferritin and transferrin were positively associated with IGM (fourth vs first sex-specific quartile: ferritin odds ratio (OR) = 2.08 (95% CI 1.43–3.04) and transferrin OR = 1.89 (95% CI 1.32–2.70)), T2DM (ferritin OR = 1.98 (95% CI 1.22–3.22) and transferrin OR = 2.42 (95% CI 1.54–3.81)), and fasting as well as 2-h glucose. TSAT (OR = 0.55 (95% CI 0.34–0.88)) and iron (OR = 0.61 (95% CI 0.38–0.97)) were inversely associated with T2DM, sTfR-F-index was inversely associated with IGM (OR = 0.67 (95% CI 0.48–0.95)). There was no strong evidence for non-linear relationships.

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Conclusions: The observed associations of several markers of iron metabolism with hyperglycemia and insulin resistance suggest that iron stores as well as iron-related metabolic pathways contribute to the pathogenesis of IGM and T2DM. Moreover, TSAT levels are decreased in T2DM patients.

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Introduction

Iron is an essential micronutrient for humans, primarily responsible for oxygen transport, electron transfer reactions and DNA synthesis. However, iron is also known to take part in the formation of highly reactive free radicals that can induce oxidative modification of various molecules (1). Since patients suffering from hereditary hemochromatosis – a disease characterized by massive iron overload – have been described to be more likely to develop type 2 diabetes mellitus (T2DM), iron overload is suspected to play a role in its etiology (2). Subsequent studies also suggested that elevated body iron stores, mainly measured by circulating ferritin levels, are associated with a higher T2DM risk in the general population (2). There is evidence that high body iron may have detrimental effects on both insulin secretion and sensitivity (1). However, more recent studies on diabetes in hereditary hemochromatosis have been inconclusive (3) and ferritin levels may be influenced by reverse causation or confounding by inflammation, as ferritin is an acute-phase protein (4).

Though investigation of additional biomarkers of iron metabolism might help elucidate the role of iron in the pathophysiology of diabetes, little is known about their association with T2DM (4). For instance, so far only three relatively small studies examined the relationship between the iron transport protein transferrin and hyperglycemia or insulin resistance in humans (5, 6, 7). These studies in general reported positive associations, although transferrin is inversely related to body iron stores and ferritin (8). In addition, while increased ferritin is quite consistently associated with hyperglycemia or insulin resistance, existing results on the soluble transferrin receptor (sTfR) and transferrin saturation (TSAT) are much more controversial (4, 9, 10, 11, 12, 13).

The aim of our investigation was to evaluate the independent associations of the markers of iron metabolism serum ferritin, transferrin, sTfR, TSAT, sTfR-to-$\log_{10}$ ferritin (sTfR-F) index, and iron with hyperglycemia and insulin resistance in the same study. As oral glucose tolerance tests (OGTTs) had been conducted in all study participants without known T2DM, we were able to investigate not only overt diabetes but also early derangements in glucose metabolism as well as the continuous traits of fasting glucose, 2-h glucose, fasting insulin, and homeostasis model assessment of insulin resistance (HOMA-IR). In addition, we assessed the shape of the observed iron marker associations in the general population and minimized potential confounding by controlling for a wide range of diabetes risk factors as well as for iron metabolism-related factors.

Subjects and methods

Subjects

The study population consisted of participants in the population-based Cooperative Health Research in the Region of Augsburg (KORA) F4 study, which was carried out in 2006–2008 as a follow-up of the KORA S4 baseline study (1999–2001). In the S4 study, 4261 participants were recruited out of a randomized two-stage cluster sample of 6640 subjects, with equal strata by sex and age, from the target population of all German residents in the region of Augsburg aged 25–74 years. The F4 study included 3080 participants (response 79.6%) (14). The current study was restricted to 2893 fasting subjects, excluding all participants with T1DM ($n=9$), drug-induced diabetes ($n=1$), unknown diabetes status ($n=84$), non-fasting ($n=13$), or missing ($n=16$) iron marker measurements or missing values in co-variables ($n=64$). For the analysis of continuous outcomes, participants taking antidiabetic medication ($n=157$) or with missing values in the continuous outcomes ($n=68$) were additionally excluded, leading to a total of $n=2674$. The investigations were carried out in accordance with the Declaration of Helsinki, including written informed consent of all participants. All study methods were approved by the Ethics Committee of the Bavarian Chamber of Physicians.

Measurements

Main outcome measurements ▸ Known type 2 diabetes (KD) was defined as self-reported and validated by questioning the responsible physician, or as current intake of antidiabetic medication. Additionally, a validation of the diabetes type was requested. If no validation but also no contradicting information was given, participants were assumed to have T2DM. All participants without known diabetes of any type underwent a standard 75-g OGTT. Blood samples were taken without stasis after an overnight fast of at least 8 h and 2 h after glucose solution intake. Serum glucose was analyzed using a hexokinase method (GLU Flex, Dade Behring, Deerfield, IL, USA). Normal glucose metabolism (NGM; fasting glucose <6.1 mmol/l and 2-h glucose <7.8 mmol/l), impaired glucose metabolism (IGM; fasting glucose ≥ 6.1 mmol/l but <7.0 mmol/l and 2-h glucose <7.8 mmol/l (i-IFG) or fasting glucose <6.1 mmol/l and 2-h glucose ≥ 7.8 mmol/l but <11.1 mmol/l (i-IGT), or both (IFG/IGT)), and newly diagnosed diabetes (NDD; fasting glucose ≥ 7.0 mmol/l or 2-h glucose ≥ 11.1 mmol/l) were diagnosed according to the 1999 WHO criteria (15). Fasting insulin from frozen serum was assessed by ELISA (Invitrogen) and HOMA-IR was calculated as fasting insulin (μU/ml) × fasting glucose (mmol/l)/22.5.

Markers of iron metabolism ▸ Biomarkers of iron metabolism were determined from frozen serum (−80 °C). Ferritin was assessed by electrochemiluminescence immunoassay (Roche Diagnostics, Mannheim, Germany), iron by colorimetry (Roche Diagnostics), sTfR by Tina-quant immunoturbidimetry (Roche Diagnostics), and transferrin by immunonephelometry (Dade Behring). TSAT and sTfR-F-index were calculated as follows: TSAT = 3.98 × serum iron (μmol/l)/transferrin (g/l) and sTfR-F-index = sTfR (mg/l)/$\log_{10}$ferritin (ng/ml).

Other laboratory measurements ▸ Total cholesterol and HDL were measured in fresh serum by enzymatic methods (CHOL Flex and AHDL Flex, Dade Behring, Marburg, Germany). Triacylglycerides were measured in fresh serum with the GPO-PAP method (Dade Behring). Non-esterified fatty acids (NEFAs) were measured in fresh plasma using an enzymatic colorimetric assay (Wako, Richmond, VA, USA). Serum creatinine was measured in fresh serum with a modified Jaffé test (KREA Flex, Dade Behring). Urinary creatinine was determined from frozen urine by nephelometry on a BN II analyzer (Siemens, Erlangen, Germany). Urinary albumin was determined from frozen urine with a modified Jaffé test (CREATININ-JK, Greiner, Bahlingen, Germany) on a Cobas Mira analyzer (Roche Diagnostics). γ-glutamyltranspeptidase was determined using the enzymatic colorimetric assay GTT-2 on a Cobas analyzer (Roche Diagnostics). High-sensitivity C-reactive protein (CRP) was determined from frozen plasma using a high-sensitivity latex enhanced nephelometric assay on a BN II analyzer (Dade Behring). Interleukin 18 (IL18) was determined from frozen plasma using the ELISA Kit from MBL (Nagoya, Japan). Leukocyte count was measured in fresh whole blood using a Coulter Counter STKS hematological analyzer.

Additional measurements and data transformations ▸ All participants underwent a standard physical and medical examination, described in detail elsewhere (16). Information about age, sex, education, menopausal status, smoking, alcohol consumption, and physical activity were assessed during a standardized interview, carried out by trained medical staff. Low education was defined as ≤ 10 years of education, regular smoking as smoking of at least one cigarette per day on average. Alcohol consumption was categorized in three groups defined as no (0 g/day), moderate (men 0.1–39.9 g/day and women 0.1–19.9 g/day), and high alcohol (men ≥ 40 g/day and women ≥ 20 g/day) consumption. Individuals who did not participate at least 1 h/week in leisure time physical training during both summer and winter were classified as physically inactive.

Statistical analysis

Differences between the groups of glucose metabolism were assessed using χ^2 tests for categorical data and ANOVAs for continuous data. Variables with skewed distributions were $\log_{10}$-transformed for further analysis and reported as geometric means with antilog of the SD.

Multinomial logistic regression analysis was performed for the outcomes IGM and T2DM vs NGM to investigate the association between iron markers and glycemic status. Iron markers were investigated as sex-specific quartiles, continuously. TSAT was additionally examined dichotomously with clinical cut-off points of 45 and 50% as suggested by a practice guideline for the diagnosis of hemochromatosis (17) and in accordance with previous studies (4, 18).

All relationships were investigated for non-linearity by testing if the addition of a non-linear spline component significantly improved the model using SAS Proc GAM. Splines are presented to illustrate non-linear relationships. Continuous outcomes were analyzed by linear regression

after *Z*-standardization of both glycemic measures and iron markers to achieve comparability despite their different scales. β-estimates with 95% CIs for *Z*-scores of glycemic traits are given per 1 s.d. change in iron markers. All analyses were stepwise multivariable adjusted. Possible interactions between sex and continuous iron markers were examined in fully adjusted models with χ^2 tests for the outcomes IGM, T2DM, and all four continuous traits.

For sensitivity analyses, we investigated i) the association of iron markers with diabetes separately for NDD and KD, ii) the effect of excluding 238 men and 110 women with anemia defined by hemoglobin levels lower than 140 and 120 g/l respectively, iii) the effect of excluding 25 participants with possible hemochromatosis (TSAT >45% and ferritin >700 ng/ml) and 70 participants with possible iron deficiency (ferritin <10 ng/ml), iv) the effect of excluding 90 participants with CRP >10 mg/l to account for acute infections/inflammation, v) the effect of further adjustment for menopausal status among women, and vi) whether the association of transferrin with IGM and T2DM was mediated by NEFAs. Significance levels were based on two-sided tests with *P* values ≤0.05 considered statistically significant. All statistical analyses were performed using SAS Software version 9.2 (Cary, NC, USA).

Results

General subject characteristics are shown by groups of glucose metabolism in Table 1. Among the 2893 participants, 479 had IGM and 315 T2DM (205 KD and 110 NDD). Transferrin and sTfR levels increased across the spectrum of NGM, IGM, and T2DM. TSAT and iron were lower in T2DM subjects compared to those with NGM and IGM. Ferritin levels were elevated in participants with IGM and T2DM compared to those with NGM. STfR-F-index was lowest among participants with IGM. All investigated iron markers were significantly correlated with each other with age- and sex-adjusted partial Pearson coefficients between 0.25 and 0.46 (not considering correlations of iron marker indices/ratios with their components), except for serum iron and transferrin, which were uncorrelated (Supplementary Table 1, see section on supplementary data given at the end of this article).

Associations between iron marker quartiles and glycemic status are shown in Table 2. Higher levels of ferritin and transferrin were associated with an increased prevalence of IGM and T2DM independently of traditional diabetes risk factors (age, sex, education, smoking status, alcohol consumption, physical activity, BMI, waist–hip ratio, actual hypertension, triacylglycerides, and total/HDL cholesterol ratio), renal function (serum creatinine and urine albumin/urine creatinine), the liver enzyme γ-glutamyltransferase, and inflammatory biomarkers (IL18, CRP, and leukocytes). Owing to the inverse correlation between ferritin and transferrin, adjusting the iron markers for each other strengthened these associations further. The IGM and T2DM odds ratios (ORs) with (95% CIs) for the fourth vs first sex-specific quartile of ferritin and transferrin ranged between 1.89 (1.32–2.70) and 2.42 (1.54–3.81). While the strong positive association between higher sTfR levels and T2DM in age- and sex-adjusted models disappeared after adjustment for other risk factors, the sTfR-F-index remained inversely associated with IGM (OR=0.67 (0.48–0.95)) and there was also a borderline independent multivariable-adjusted association of the sTfR-F-index with T2DM (OR=0.65 (0.42–1.01), *P* value for linear relation was 0.04). TSAT (OR=0.55 (0.34–0.88)) and iron (OR=0.61 (0.38–0.97)) were inversely associated with T2DM but not with IGM. Further adjusting for NEFAs attenuated the associations of ferritin and transferrin with IGM and T2DM slightly, while the associations between TSAT and iron with T2DM were strengthened (Supplementary Table 2, see section on supplementary data given at the end of this article). When investigating TSAT as a dichotomized variable with a clinical cut-off point of 45% (434 participants with elevated TSAT), the inverse association between TSAT and T2DM was confirmed (OR=0.605 (0.367–0.998), Supplementary Table 3). Whereas ferritin, transferrin, and sTfR-F-index were slightly stronger associated with NDD than with KD, TSAT, and iron were only associated with KD (Supplementary Table 4). There was no evidence for an interaction between sex and iron markers in the IGM or T2DM associations (*P*>0.2) and a non-linear relationship was only suggested for transferrin and T2DM (Supplementary Figure 1 and Supplementary Table 5). The spline showed a positive linear association between the 10th and the 95th transferrin percentile and an inverse association at the extremes (*P*=0.01). However, statistical significance for a non-linear association disappeared when we excluded the participants with the lowest and highest 0.5% of transferrin levels (*P*=0.26).

When analyzing the continuous outcomes, we found higher levels of ferritin to be independently associated with increased levels of fasting glucose, 2-h glucose, fasting insulin, and HOMA-IR (Fig. 1). The association with 2-h glucose was observed in both men and women but was stronger in men (Supplementary Table 6, see section on supplementary data given at the end of this article). Transferrin was positively associated with

Table 1 General characteristics of the study population ($n=2893$).

Characteristics	Glucose metabolism status			P
	NGM ($n=2099$)	IGM ($n=479$)	Type 2 diabetes ($n=315$)	
Age (years)	52.8±12.6	63.2±10.9	67.1±9.1	<0.0001
Sex, male (%)	45.5	52.6	60.6	<0.0001
Postmenopausal status among women[a] (%)	42.4	74.9	80.6	<0.0001
Education ≤10 years (%)	40.5	52.6	60.6	<0.0001
Smoking (%)				
Current	20.6	8.8	12.4	<0.0001
Former	35.7	42.6	45.1	
Never	43.7	48.6	42.5	
Alcohol intake (%)				
0 g/day	28.7	30.3	37.5	0.0222
Men 0.1–39.9 g/day and women 0.1–19.9 g/day	54.0	50.7	46.0	
Men ≥40 g/day and women ≥20 g/day	17.3	19.0	16.5	
Physically inactive (%)	41.7	50.5	59.4	<0.0001
BMI (kg/m^2)	26.6±4.3	29.8±4.8	31.2±5.0	<0.0001
Waist–hip ratio (units)	0.86±0.08	0.92±0.08	0.95±0.07	<0.0001
Actual hypertension (%)	20.6	46.8	73.3	<0.0001
Triacylglycerides[A] (mmol/l)	1.09±1.69	1.49±1.65	1.67±1.76	<0.0001
Total/HDL cholesterol ratio[A] (units)	3.79±1.32	4.29±1.30	4.35±1.32	<0.0001
Serum creatinine[A] (μmol/l)	76.8±1.2	80.4±1.2	86.0±1.3	<0.0001
Urine albumin/urine creatinine[A] (mg/g)	6.40±2.53	8.55±2.99	14.08±4.13	<0.0001
γ-glutamyltransferase[A] (μkat/l)	0.43±1.93	0.62±2.07	0.68±2.02	<0.0001
Interleukin 18[A] (pg/ml)	277.5±1.5	317.1±1.5	344.1±1.5	<0.0001
C-reactive protein[A] (mg/l)	1.03±2.93	1.82±2.83	2.15±2.96	<0.0001
Leukocytes[A] (1/nl)	5.59±1.30	5.94±1.30	6.40±1.30	<0.0001
Non-esterified fatty acids (mmol/l)	0.23±0.09	0.28±0.09	0.28±0.10	<0.0001
Fasting glucose[A,b] (mmol/l)	5.08±1.09	5.70±1.12	6.90±1.23	<0.0001
2-h glucose[A,c] (mmol/l)	5.29±1.25	8.22±1.22	11.87±1.29	<0.0001
Fasting insulin[A,d] (μU/ml)	3.91±2.40	6.97±2.41	9.08±2.62	<0.0001
HOMA-IR[A,e]	0.88±2.46	1.77±2.49	2.78±2.69	<0.0001
Ferritin[A] (ng/ml)	102.8±2.8	170.6±2.6	169.0±2.7	<0.0001
Transferrin (g/l)	2.54±0.37	2.56±0.36	2.62±0.38	0.0015
Soluble transferrin receptor[A] (mg/l)	2.89±1.31	2.97±1.30	3.15±1.37	<0.0001
Transferrin saturation (%)	34.0±12.4	34.0±11.9	30.5±11.0	<0.0001
sTfR-to-log$_{10}$ferritin-index[A] (units)	1.48±1.57	1.36±1.47	1.45±1.60	0.0006
Iron (μmol/l)	21.3±7.2	21.5±6.9	19.7±6.6	0.0004

HOMA-IR, homeostasis model assessment of insulin resistance; IGM, impaired glucose metabolism; NGM, normal glucose metabolism. Percentages are given for categorical variables, arithmetic or geometric[A] means and S.D.s or antilog[A] of S.D.s for continuous variables. In total, 2893 individuals were investigated, except for postmenopausal status, where [a]$n=1495$ women were investigated, and the continuous glucose and insulin traits where individuals with missing values or taking antidiabetic medication were excluded: [b]$n=2722$, [c]$n=2677$, [d]$n=2719$, and [e]$n=2719$.

fasting and 2-h glucose. sTfR was inversely associated with fasting glucose and positively with fasting insulin and HOMA-IR. TSAT and iron were inversely associated with fasting glucose. The sTfR-F-index was inversely associated with fasting and 2-h glucose. The inverse associations between sTfR and sTfR-F-index and fasting glucose were stronger and statistically significant only in men.

Our sensitivity analyses showed that the associations were largely independent of hemoglobin-defined anemia, of possible hemochromatosis or iron deficiency, or of possible acute infections and inflammation. However, the positive association of transferrin and the inverse association of TSAT with HOMA-IR became statistically significant only when excluding anemic participants (Supplementary Tables 2 and 6). Moreover, adjustment for menopausal status did not appreciably change the results among women (data not shown).

Discussion

Our main findings and possible linked pathomechanisms and conclusions respectively are summarized in Table 3.

Ferritin and transferrin

In the past, most studies on biomarkers of iron status and T2DM investigated circulating ferritin levels as the major iron storage protein in blood and found that increased

Table 2 Association between markers of iron metabolism and impaired glucose metabolism ($n=479$) or type 2 diabetes ($n=315$) vs normal glucose metabolism ($n=2099$). All results come from multivariable, multinomial logistic regression analysis. Exposure quartiles were transformed sex-specifically. Co-variables were categorically, log- or untransformed as described in Table 1.

Iron markers and adjustment	Model	ORs (95% CI) for impaired glucose metabolism				ORs (95% CI) for type 2 diabetes			
		Quartile 2	Quartile 3	Quartile 4	*P**	Quartile 2	Quartile 3	Quartile 4	*P**
Ferritin (ng/ml)		M: >124.5–209.0	M: >209.0–339.2	M: >339.2		M: >124.5–209.0	M: >209.0–339.2	M: >339.2	
adjusted for		F: >42.7–83.5	F: >83.5–143.1	F: >143.1		F: >42.7–83.5	F: >83.5–143.1	F: >143.1	
Age and sex	1	1.29 (0.91–1.82)	1.59 (1.14–2.21)‡	2.92 (2.13–3.99)§	<0.0001	1.24 (0.82–1.88)	1.49 (1.01–2.20)†	2.26 (1.56–3.28)§	<0.0001
+ Risk factors	2	1.18 (0.82–1.70)	1.28 (0.90–1.81)	1.74 (1.23–2.44)‡	0.0023	1.29 (0.81–2.06)	1.38 (0.89–2.16)	1.46 (0.95–2.25)	0.0780
+ Iron markers	3	1.32 (0.90–1.93)	1.46 (1.00–2.12)†	2.08 (1.43–3.04)§	<0.0001	1.56 (0.96–2.56)	1.74 (1.08–2.80)†	1.98 (1.22–3.22)‡	0.0015
Transferrin (g/l)		M: >2.28–2.48	M: >2.48–2.72	M: >2.72		M: >2.28–2.48	M: >2.48–2.72	M: >2.72	
adjusted for		F: >2.34–2.54	F: >2.54–2.80	F: >2.80		F: >2.34–2.54	F: >2.54–2.80	F: >2.80	
Age and sex	1	1.20 (0.89–1.63)	1.56 (1.16–2.11)‡	1.87 (1.38–2.53)§	0.0002	1.18 (0.80–1.75)	1.86 (1.27–2.71)‡	3.10 (2.15–4.47)§	<0.0001
+ Risk factors	2	1.09 (0.79–1.49)	1.29 (0.94–1.77)	1.39 (1.00–1.93)†	0.1388	0.98 (0.63–1.52)	1.40 (0.92–2.13)	1.79 (1.18–2.71)‡	0.0228
+ Iron markers	3	1.16 (0.84–1.60)	1.48 (1.07–2.05)†	1.89 (1.32–2.70)§	0.0010	1.05 (0.67–1.63)	1.61 (1.05–2.47)†	2.42 (1.54–3.81)§	0.0004
sTfR (mg/l)		M: >2.46–2.88	M: >2.88–3.37	M: >3.37		M: >2.46–2.88	M: >2.88–3.37	M: >3.37	
adjusted for		F: >2.43–2.85	F: >2.85–3.40	F: >3.40		F: >2.43–2.85	F: >2.85–3.40	F: >3.40	
Age and sex	1	0.81 (0.60–1.10)	1.01 (0.75–1.35)	1.11 (0.83–1.49)	0.3029	1.13 (0.77–1.67)	1.09 (0.73–1.62)	1.73 (1.19–2.51)‡	0.0006
+ Risk factors	2	0.73 (0.52–1.01)	0.82 (0.59–1.13)	0.78 (0.56–1.09)	0.3062	1.01 (0.65–1.56)	0.88 (0.56–1.36)	0.98 (0.64–1.52)	0.9432
+ Iron markers	3	0.73 (0.53–1.02)	0.85 (0.61–1.17)	0.83 (0.59–1.18)	0.6719	1.03 (0.66–1.59)	0.90 (0.58–1.41)	1.02 (0.65–1.60)	0.8236
TSAT (%)		M: >27.7–34.2	M: >34.2–42.1	M: >42.1		M: >27.7–34.2	M: >34.2–42.1	M: >42.1	
adjusted for		F: >24.1–31.1	F: >31.1–38.5	F: >38.5		F: >24.1–31.1	F: >31.1–38.5	F: >38.5	
Age and sex	1	1.09 (0.80–1.47)	1.04 (0.77–1.40)	0.97 (0.71–1.31)	0.6299	0.73 (0.52–1.02)	0.46 (0.32–0.65)§	0.42 (0.29–0.61)§	<0.0001
+ Risk factors	2	1.15 (0.84–1.58)	1.22 (0.89–1.68)	1.35 (0.97–1.86)	0.2235	0.81 (0.55–1.18)	0.64 (0.43–0.95)†	0.69 (0.45–1.07)	0.0065
+ Iron markers	3	1.03 (0.74–1.44)	1.08 (0.77–1.51)	1.15 (0.80–1.66)	0.9543	0.69 (0.47–1.03)	0.54 (0.35–0.82)‡	0.55 (0.34–0.88)†	0.0002
sTfR-F-index (units)		M: >1.04–1.26	M: >1.26–1.53	M: >1.53		M: >1.04–1.26	M: >1.26–1.53	M: >1.53	
adjusted for		F: >1.21–1.49	F: >1.49–1.96	F: >1.96		F: >1.21–1.49	F: >1.49–1.96	F: >1.96	
Age and sex	1	0.89 (0.67–1.18)	0.63 (0.47–0.85)‡	0.76 (0.57–1.03)	0.0133	0.85 (0.59–1.21)	0.69 (0.48–0.99)†	0.96 (0.68–1.37)	0.5990
+ Risk factors	2	0.93 (0.68–1.25)	0.64 (0.46–0.88)‡	0.77 (0.56–1.06)	0.0404	0.97 (0.65–1.45)	0.74 (0.49–1.06)	0.80 (0.53–1.21)	0.4310
+ Iron markers	3	0.90 (0.66–1.22)	0.61 (0.44–0.84)‡	0.67 (0.48–0.95)†	0.0030	0.93 (0.62–1.39)	0.69 (0.45–1.05)	0.65 (0.42–1.01)	0.0445
Iron (μmol/l)		M: >17.4–21.4	M: >21.4–26.3	M: >26.3		M: >17.4–21.4	M: >21.4–26.3	M: >26.3	
adjusted for		F: >15.7–19.6	F: >19.6–24.2	F: >24.2		F: >15.7–19.6	F: >19.6–24.2	F: >24.2	
Age and sex	1	0.86 (0.64–1.16)	0.79 (0.58–1.06)	1.13 (0.84–1.51)	0.2560	0.81 (0.58–1.13)	0.60 (0.42–0.86)‡	0.58 (0.40–0.85)‡	0.0004
+ Risk factors	2	0.88 (0.64–1.21)	0.83 (0.60–1.15)	1.35 (0.98–1.86)	0.0329	0.88 (0.60–1.29)	0.70 (0.47–1.05)	0.74 (0.48–1.16)	0.0508
+ Iron markers	3	0.81 (0.58–1.11)	0.73 (0.52–1.02)	1.14 (0.81–1.61)	0.2682	0.80 (0.54–1.18)	0.61 (0.40–0.92)†	0.61 (0.38–0.97)†	0.0044

M, male; F, female. **P* values for linear relation using the continuous iron marker variables as exposure: $^{\dagger}P<0.05$; $^{\ddagger}P<0.01$; and $^{\S}P<0.001$. Model 1, adjusted for age and sex; model 2, model 1+ education, smoking status, alcohol consumption, physical inactivity, BMI, waist–hip ratio, actual hypertension, triacylglycerides, total/HDL cholesterol ratio, serum creatinine, urine albumin/urine creatinine, γ-glutamyltransferase, interleukin 18, C-reactive protein, and leukocytes; model 3, model 2+ other iron markers (ferritin for transferrin, sTfR; transferrin for ferritin, sTfR; sTfR for ferritin, transferrin; TSAT for ferritin, sTfR; sTfR-F-index for transferrin; iron for ferritin, transferrin, sTfR).

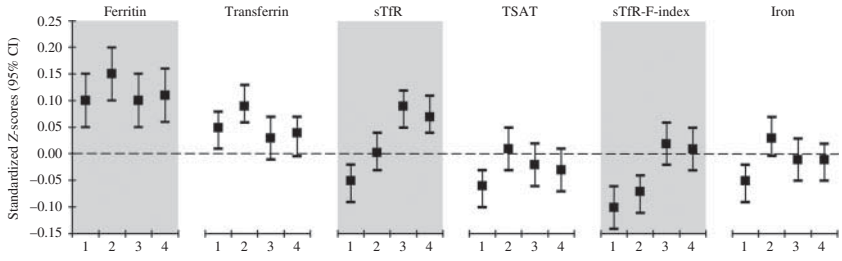

Figure 1

Estimated difference in continuous glycemic and insulin outcomes (1, fasting glucose; 2, 2-h glucose; 3, fasting insulin; and 4, homeostasis model assessment of insulin resistance) in 2674 subjects that did not take antidiabetic medication; expressed as s.d. change in the respective continuous outcome (standardized *Z*-score with 95% CI) according to a 1 s.d. change in the respective iron marker, adjusted on model 3 level.

ferritin levels are associated with T2DM (4) and earlier derangements in glucose metabolism (19, 20, 21). Our ferritin results, which were adjusted for a multitude of possible confounders, are in line with these findings and additionally show that the estimated strength of the association is very similar between subjects with T2DM and IGM and stronger in newly diagnosed than in known diabetic cases, suggesting that ferritin or rather increased body iron might play a role in the early T2DM pathogenesis. In line with these findings, our study revealed that higher levels of ferritin were associated with increased fasting glucose, fasting insulin, HOMA-IR, and especially 2-h glucose.

Transferrin has been much less investigated. The iron transport protein is known to be synthesized increasingly if body iron stores are low, and thus transferrin is inversely correlated with ferritin. Nevertheless, we observed strong positive associations between higher transferrin levels and both IGM and T2DM, which were independent of all other investigated risk factors. Consistently, we observed higher levels of fasting and 2-h glucose. Two smaller studies in 1277 French and 494 Dutch subjects previously reported

Table 3 Main association results of increased serum iron marker levels in the KORA F4 study with interpretation or conclusion.

Iron marker	**Main findings** (with respect to increased serum levels (multivariable adjusted))	**Function in iron metabolism** (↑, levels increase and ↓, levels decrease if body iron status is high)	**Possible interpretation or conclusion**
Ferritin	Positively associated with all investigated T2D-related traits, especially with NDD as well as 2-h glucose	Main iron storage protein ↑	Increased body iron might play a role in the early T2D pathogenesis (1, 2)
Transferrin	Positively associated with IGM, T2D, fasting glucose, and especially 2-h glucose	Iron transport protein ↓	Antagonistic effect on insulin action (22) Transferrin might alternatively be upregulated due to glycation (27)
TSAT	Inversely associated with KD and fasting glucose	Transferrin proportion saturated with iron ↑	Strongly correlated with iron
Iron	Inversely associated with KD and fasting glucose	Non-haeme iron, mainly bound to transferrin ↑	Upregulated hepcidin might cause reduced serum iron (32) Upregulation might be cytokine-induced (33) or occur through increased iron-bound transferrin (33) or insulin (34) levels
sTfR	Inversely associated with fasting glucose; positively associated with fasting insulin and HOMA-IR	The part of the transferrin receptor that is released into the circulation during endocytosis of the transferrin–iron complex ↓	Increased sTfR might be caused by high insulin levels (38)
sTfR-F-index	Inversely associated with IGM, T2D, fasting glucose, and 2-h glucose	Indicator of body iron status ↓	Usefulness questioned in insulin-resistant states

HOMA-IR, homeostasis model assessment of insulin resistance; KD, known diabetes; NDD, newly diagnosed diabetes.

that increased transferrin levels were associated with higher fasting insulin, 2-h glucose, and HOMA-IR. These studies also described a higher risk to develop incident hyperglycemia and T2DM with increased baseline transferrin levels, which is consistent with our observation of increased transferrin levels already seen in IGM (5, 7).

The strong positive association of transferrin with glycemic outcomes despite its inverse correlation with ferritin suggests an association of transferrin with IGM and T2DM through an iron-independent mechanism, which is, however, still largely unknown. Experiments in rats showed that transferrin has an antagonistic effect on insulin action and might thereby contribute to insulin resistance (22). Another mechanism might be an enhanced production of NEFAs as transferrin was found to increase the lipolytic activity of adipocytes (23). Elevated NEFAs may contribute to insulin resistance by opposing insulin action, increasing hepatic glucose output and exerting detrimental effects on the pancreas (24, 25). Consistent with the observations in adipocytes, transferrin was positively associated with NEFAs and adipocyte insulin resistance in 492 individuals with or at an increased risk of T2DM and cardiovascular diseases (26). However, although transferrin was also positively associated with elevated NEFAs in our study, the NEFA levels did not mediate a major part of the observed association. Alternatively, one could speculate that transferrin levels are upregulated in order to compensate the compromised action resulting from high glucose levels, as it has recently been shown that transferrin is highly susceptible to glycation and that spots are modified that are particularly relevant to its function (27).

TSAT and iron

We found TSAT to be inversely associated with fasting glucose levels as well as T2DM, and here specifically with previously known diabetes, after multivariable adjustment including ferritin and sTfR. There was no evidence for an association between TSAT and 2-h glucose or IGM, which was diagnosed in more than half of IGM subjects only via increased 2-h glucose levels. The TSAT was more strongly correlated with iron (Pearson $r=0.92$) than with transferrin ($r=-0.39$) and overall our TSAT association results were very similar to serum iron.

The multivariable adjusted inverse association between TSAT and T2DM seen in our study is in line with the results of our previous meta-analysis, which has summarized the mainly unadjusted published results on TSAT and prevalent T2DM (4). Likewise, independent inverse associations with prevalent diabetes were meanwhile reported in an Australian study of 1834 Caucasian men but not 2351 women (28), and with a status of impaired fasting glucose in 6451 and 2413 participants of the US (19) and South Korean (20) NHANES studies respectively. Interestingly, all these results are in sharp contrast with one cross-sectional and two prospective Danish studies in which sex-adjusted TSAT levels above vs below 50% were associated with an increased risk of T2DM (18). Up to now, only one other study has investigated TSAT and incident T2DM, using cut-off levels of 50%, but did not find evidence for association (4, 29). In the present study, 8.4% of subjects had TSAT levels above 50% and the effect estimate non-significantly pointed in the same inverse direction as the effect estimate for the fourth vs first TSAT quartile.

Several mechanisms might explain the low TSAT values seen in KD subjects of the present study. Thomas *et al*. (30) showed that USA diabetic outpatients had a higher prevalence of TSAT values below 20% than subjects from the US general population (NHANES III), especially when the glomerular filtration rate was below 60 ml/min per 1.73 m^2. However, in our study the TSAT–KD association was independent of serum creatinine and urine albumin/urine creatinine, two alternative markers of renal function. Moreover, Thomas *et al*. (30) reported a strong association between anemia and diabetes, which, however, was not observed in a previous investigation of the KORA F4 study (31). Consequently, the TSAT–T2DM association in the present investigation was also not explained by a different anemia prevalence between T2DM and normoglycemic subjects as shown in the hemoglobin sensitivity analysis. Alternatively, the low TSAT levels in T2DM subjects might be caused by up-regulated hepcidin, which, as hepcidin is the key regulator of systemic iron levels, would lead to reduced circulating serum iron (32). Hepcidin upregulation might be cytokine-induced due to the subclinical inflammatory state typically observed in T2DM (32, 33). In fact, we observed inverse correlations of CRP and leukocytes with TSAT, but the inverse association between TSAT and T2DM was only slightly attenuated by adjustment for these two inflammatory biomarkers as well as IL18. Residual confounding might be an issue here, as we did not have data on additional cytokines available in our study. Hepcidin might also be upregulated in T2DM subjects through increased iron-bound transferrin (33) and insulin (34) levels, among others. However, it should be noted that although a previous study of 135 men had observed elevated prohepcidin levels in 18 T2DM vs 92 normoglycemic men (35), there was no association between hepcidin and CRP levels or T2DM in 1259 Han

Chinese (34), which might be due to rapid hepcidin clearing from the circulation (33).

sTfR and sTfR-F-index

The sTfR is the part of the transferrin receptor that is separated and released into the circulation during endocytosis of the transferrin–iron complex. Reflecting erythropoietic activity of bone marrow under conditions of sufficient iron stores, sTfR levels increase proportional to iron requirements if iron stores are low (36). As expected, we observed a positive correlation between circulating sTfR and transferrin and an inverse one with ferritin. Although individuals with increased circulating sTfR had significantly higher fasting insulin and HOMA-IR values, there was no evidence for an association with IGM, T2DM, and 2-h glucose, but an inverse association with fasting glucose levels, which was restricted to the male study participants.

Recent studies investigating association between sTfR and risk of T2DM were controversial (10, 11, 13, 37) and our previous meta-analysis had also revealed strong heterogeneity between the published study results (4). This might partly be due to different levels of co-variable adjustment as sTfR concentrations are strongly determined also by other factors than iron status. In fact, we observed that higher sTfR levels were associated with increased T2DM prevalence in unadjusted analyses but not after controlling for important diabetes risk factors. The finding of strong sTfR association with fasting insulin and HOMA-IR without finding consistent associations with hyperglycemia suggests a reverse causation scenario, with high sTfR levels being caused by high insulin levels in an insulin-resistant state. This would be in line with the observation of Fernandez-Real *et al*. (38), who observed a decrease in sTfR concentration after exercise-induced improvement of insulin sensitivity in 26 obese women.

In addition, our study revealed strong inverse associations of sTfR-F-index with T2DM and IGM as well as fasting and 2-h glucose, but not with fasting insulin or HOMA-IR. To our knowledge, the sTfR-F has not yet been investigated for associations with any glycemic or insulin outcomes, except for a small correlation study in 110 female T2DM patients from Kuwait (39). Importantly, the sTfR-F-index may not be confused with the sTfR-F-ratio, which lacks the $\log_{10}$-transformation of ferritin in the denominator and that has been investigated for association with T2DM in several studies, all reporting inverse associations (11, 13, 37, 40, 41). This ratio in our as in other studies (11, 13) showed an extremely strong correlation (Pearson $r=-0.97$) with ferritin, questioning its additional value. The sTfR-F-index in contrast is considered to be a useful indicator of body iron over a wide range of normal and depleted iron stores, with decreased values reflecting increased body iron (36, 42). However, our observation that insulin resistance might lead to increased sTfR levels also suggests that the usefulness of the sTfR-F-index as an indicator of body iron is compromised in insulin-resistant states, which might be the reason why we did not observe an association between the sTfR-F-index and fasting insulin or HOMA-IR.

Strengths and limitations

To our knowledge, this study is the first to investigate the association of iron stores and iron metabolism with IGM and T2DM using ferritin, transferrin, sTfR, TSAT, and sTfR-F-index simultaneously to better approximate the iron status and related metabolic processes through multiple pathways. The main limitation of our study is its cross-sectional design, not allowing conclusions on causality. However, we compared both participants with IGM as well as manifest T2DM with normoglycemic participants in order to contribute to the clarification of the temporal relationships. It should also be pointed out that, despite the comparatively high response rate, the generalizability of the KORA F4 follow-up results is somewhat reduced, because a rather healthy part of the general population participated. Important strengths are our large population-based study, the availability of fasting serum levels and that we adjusted our analyses for a wide range of traditional and novel diabetes risk factors and iron marker-related factors. Moreover, we performed several sensitivity analyses to test the robustness of our results. Nevertheless, unknown or unmeasured diabetes and iron-associated factors could have confounded our findings.

Overall, we have clearly shown that several markers of iron metabolism are independently associated with IGM and T2DM as well as continuous glycemic traits. High ferritin and transferrin levels as well as low sTfR-F-index were consistently associated with an increased prevalence of IGM and T2DM. While increased ferritin and decreased sTfR-F-index probably reflect elevated iron stores, the association of transferrin with diabetes seems to be driven by an as yet unknown mechanism independent of iron stores, inflammation, or lipid metabolism. Body iron stores and related metabolic processes thus might contribute to the pathogenesis of IGM and T2DM, though a reverse causation scenario cannot be excluded. In addition to the pathophysiological considerations, the knowledge of downregulated

TSAT levels in T2DM is also clinically important because TSAT cut-off levels established in the general population for iron status evaluation might not be useful for T2DM patients.

Supplementary data

This is linked to the online version of the paper at http://dx.doi.org/10.1530/EJE-15-0631.

Declaration of interest

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

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Author contribution statement

C Huth and S Beuerle had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. M Heier, C Herder, W Rathmann, C Meisinger, A Peters, and B Thorand contributed to the conception and design of the KORA F4 study. C Huth, M Heier, C Herder, W Koenig, F Kronenberg, K Oexle, W Rathmann, J Seissler, D Stöckl, C Meisinger, A Peters, and B Thorand were responsible for the acquisition of data. C Huth and S Beuerle analyzed and interpreted the data and wrote the manuscript. A Zierer and B Thorand advised the statistical analyses. All authors contributed to data interpretation, critically reviewed and edited the manuscript and approved the version to be published.

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