



# Association of Multiple Biomarkers of Iron Metabolism and Type 2 Diabetes: The EPIC-InterAct Study

*Diabetes Care* 2016;39:572–581 | DOI: 10.2337/dc15-0257

## OBJECTIVE

Observational studies show an association between ferritin and type 2 diabetes (T2D), suggesting a role of high iron stores in T2D development. However, ferritin is influenced by factors other than iron stores, which is less the case for other biomarkers of iron metabolism. We investigated associations of ferritin, transferrin saturation (TSAT), serum iron, and transferrin with T2D incidence to clarify the role of iron in the pathogenesis of T2D.

## RESEARCH DESIGN AND METHODS

The European Prospective Investigation into Cancer and Nutrition–InterAct study includes 12,403 incident T2D cases and a representative subcohort of 16,154 individuals from a European cohort with 3.99 million person-years of follow-up. We studied the prospective association of ferritin, TSAT, serum iron, and transferrin with incident T2D in 11,052 cases and a random subcohort of 15,182 individuals and assessed whether these associations differed by subgroups of the population.

## RESULTS

Higher levels of ferritin and transferrin were associated with a higher risk of T2D (hazard ratio [HR] [95% CI] in men and women, respectively: 1.07 [1.01–1.12] and 1.12 [1.05–1.19] per 100  $\mu\text{g/L}$  higher ferritin level; 1.11 [1.00–1.24] and 1.22 [1.12–1.33] per 0.5 g/L higher transferrin level) after adjustment for age, center, BMI, physical activity, smoking status, education, hs-CRP, alanine aminotransferase, and  $\gamma$ -glutamyl transferase. Elevated TSAT ( $\geq 45\%$  vs.  $< 45\%$ ) was associated with a lower risk of T2D in women (0.68 [0.54–0.86]) but was not statistically significantly associated in men (0.90 [0.75–1.08]). Serum iron was not associated with T2D. The association of ferritin with T2D was stronger among leaner individuals ( $P_{\text{interaction}} < 0.01$ ).

## CONCLUSIONS

The pattern of association of TSAT and transferrin with T2D suggests that the underlying relationship between iron stores and T2D is more complex than the simple link suggested by the association of ferritin with T2D.

Clara Podmore,<sup>1</sup> Karina Meidtnier,<sup>2</sup> Matthias B. Schulze,<sup>2</sup> Robert A. Scott,<sup>1</sup> Anna Ramond,<sup>3</sup> Adam S. Butterworth,<sup>3</sup> Emanuele Di Angelantonio,<sup>3</sup> John Danesh,<sup>3</sup> Larraitz Arriola,<sup>4,5,6</sup> Aurelio Barricarte,<sup>6,7</sup> Heiner Boeing,<sup>8</sup> Françoise Clavel-Chapelon,<sup>9,10</sup> Amanda J. Cross,<sup>11</sup> Christina C. Dahm,<sup>12</sup> Guy Fagherazzi,<sup>9,10</sup> Paul W. Franks,<sup>13,14</sup> Diana Gavrila,<sup>6,15</sup> Sara Grioni,<sup>9,10</sup> Marc J. Gunter,<sup>11</sup> Gaëlle Gusto,<sup>9,10</sup> Paula Jakyszyn,<sup>17</sup> Verena Katzke,<sup>18</sup> Timothy J. Key,<sup>19</sup> Tilman Kühn,<sup>18</sup> Amalia Mattiello,<sup>20</sup> Peter M. Nilsson,<sup>13</sup> Anja Olsen,<sup>21</sup> Kim Overvad,<sup>12,22</sup> Domenico Palli,<sup>23</sup> J. Ramón Quirós,<sup>24</sup> Olov Rolandsson,<sup>14</sup> Carlotta Sacerdote,<sup>25,26</sup> Emilio Sánchez-Cantalejo,<sup>6,27</sup> Nadia Slimani,<sup>28</sup> Iyonna Sluijs,<sup>29</sup> Annemieke M.W. Spijkerman,<sup>30</sup> Anne Tjønneland,<sup>21</sup> Rosario Tumino,<sup>31,32</sup> Daphne L. van der A,<sup>30</sup> Yvonne T. van der Schouw,<sup>29</sup> Edith J.M. Feskens,<sup>33</sup> Nita G. Forouhi,<sup>1</sup> Stephen J. Sharp,<sup>1</sup> Elio Riboli,<sup>11</sup> Claudia Langenberg,<sup>1</sup> and Nicholas J. Wareham<sup>1</sup>

<sup>1</sup>MRC Epidemiology Unit, Institute of Metabolic Science, University of Cambridge School of Clinical Medicine, Cambridge, U.K.

<sup>2</sup>Department of Molecular Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany

<sup>3</sup>Department of Public Health and Primary Care, University of Cambridge, Cambridge, U.K.

<sup>4</sup>Public Health Division of Gipuzkoa, Basque Government, San Sebastian, Spain

<sup>5</sup>Instituto Bío-Donostia, Basque Government, San Sebastian, Spain

<sup>6</sup>Consortium for Biomedical Research in Epidemiology and Public Health (CIBER Epidemiología y Salud Pública), Madrid, Spain

<sup>7</sup>Navarre Public Health Institute, Pamplona, Navarra, Spain

<sup>8</sup>Department of Epidemiology, German Institute of Human Nutrition Potsdam-Rehbruecke, Nuthetal, Germany

<sup>9</sup>INSERM, CESP Centre for Research in Epidemiology and Population Health, Villejuif, France

<sup>10</sup>University Paris-Sud, Villejuif, France

<sup>11</sup>Department of Epidemiology & Biostatistics, School of Public Health, Imperial College London, London, U.K.

<sup>12</sup>Department of Public Health, Section for Epidemiology, Aarhus University, Aarhus, Denmark

Hereditary hemochromatosis (HHC), a genetic disorder characterized by systemic iron overload, is reported to be associated with diabetes (1). Similarly, an overrepresentation of diabetes cases has also been described among individuals with conditions of acquired iron overload, such as thalassemia major (2). This raises the question whether high levels of body iron are a risk factor for type 2 diabetes in the general population; this would have implications for the prevention and treatment of type 2 diabetes. Cross-sectional and prospective population studies report a positive association between ferritin and type 2 diabetes (3,4). However, although ferritin is considered a marker of iron stores in healthy individuals (5–7), it is also an acute phase reactant and is influenced by inflammation, liver disease, and insulin resistance, which are also associated with type 2 diabetes (8–11).

Other commonly measured biomarkers of iron metabolism reflect different aspects of the process and are less influenced by the above-mentioned conditions; therefore, their use may provide additional information on the role of iron in the pathogenesis of type 2 diabetes. Transferrin is the iron-binding protein in circulation, and its levels rise with increasing iron requirements. Serum iron is difficult to interpret in isolation because it

has a diurnal variation and hence varies significantly without changes in total body iron (12). Transferrin saturation (TSAT) is the proportion of transferrin bound to serum iron and is in part a marker of iron absorption: it reflects the proportion of circulating iron in the context of iron requirements. TSAT is elevated in the presence of non-transferrin-bound iron, which in turn is responsible for iron-related oxidative damage (13,14).

We investigated the association of ferritin, TSAT, serum iron, and transferrin with incident type 2 diabetes in a large, prospective European case-cohort study. We also assessed whether these associations have a threshold effect or differ by subgroups of the population, such as individuals not presenting signs of conditions commonly associated with hyperferritinemia.

## RESEARCH DESIGN AND METHODS

### The EPIC-InterAct Study

#### Participants and Study Design

The InterAct study is a large case-cohort study of incident type 2 diabetes and is nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) study, the design and population characteristics of which have been published previously (15). In brief, a total of 12,403 incident cases of type 2 diabetes were ascertained and verified during 3.99 million person-years of follow-up (mean follow-up, 11.7 years) of 340,234

eligible EPIC participants (men and women, age 20–80 years at baseline, who had a stored blood sample and a reported diabetes status). The subcohort ( $n = 16,154$ ), which was a representative sample of the original cohort, was identified by randomly selecting individuals from each center. We excluded individuals who had prevalent clinically diagnosed diabetes at baseline. By design, there are individuals with incident diabetes who were also randomly allocated to the subcohort ( $n = 778$ ), and these are included as cases in case-cohort analyses (15). A detailed breakdown of participants with data on the iron biomarkers and covariates are detailed in RESULTS. Participants gave written informed consent, and the study was approved by the local ethics committee in the participating countries and the internal review board of the International Agency for Research on Cancer.

#### Measurements

Standardized information was collected by questionnaire and physical examination at recruitment as part of EPIC. Participants were asked about their level of education, smoking status, and alcohol consumption (which was subsequently converted into mean grams/day). Diet and physical activity were assessed using questionnaires (15,16). Most cohorts were asked about the presence of a family history of type 2 diabetes, defined as type 2 diabetes in a first-degree relative, except those in Italy, Spain,

<sup>13</sup>Department of Clinical Sciences, Clinical Research Center, Skåne University Hospital, Lund University, Malmö, Sweden

<sup>14</sup>Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden

<sup>15</sup>Department of Epidemiology, Murcia Regional Health Council, Murcia, Spain

<sup>16</sup>Fondazione IRCCS Istituto Nazionale dei Tumori Milan, Milan, Italy

<sup>17</sup>Nutrition, Environment and Cancer Unit, Department of Epidemiology, Catalan Institute of Oncology (ICO), Bellvitge Biomedical Research Institute (IDIBELL), Barcelona, Spain

<sup>18</sup>Division of Cancer Epidemiology, German Cancer Research Centre (DKFZ), Heidelberg, Germany

<sup>19</sup>Cancer Epidemiology Unit, Nuffield Department of Population Health, University of Oxford, Oxford, U.K.

<sup>20</sup>Dipartimento di Medicina Clinica e Chirurgia, Federico II University, Naples, Italy

<sup>21</sup>Danish Cancer Society Research Center, Copenhagen, Denmark

<sup>22</sup>Department of Cardiology, Aalborg University Hospital, Aalborg, Denmark

<sup>23</sup>Molecular and Nutritional Epidemiology Unit, Cancer Research and Prevention Institute (ISPO), Florence, Italy

<sup>24</sup>Consejería de Sanidad, Public Health Directorate, Oviedo-Asturias, Spain

<sup>25</sup>Unit of Cancer Epidemiology, AO Citta' della Salute e della Scienza Hospital—University of Turin and Center for Cancer Prevention (CPO), Turin, Italy

<sup>26</sup>Human Genetics Foundation (HuGeF), Turin, Italy

<sup>27</sup>Escuela Andaluza de Salud Pública, Instituto de Investigación Biosanitaria ibs.Granada, Hospitales Universitarios de Granada/Universidad de Granada, Granada, Spain

<sup>28</sup>International Agency for Research on Cancer, Dietary Exposure Assessment Group (DEX), Lyon, France

<sup>29</sup>Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht, the Netherlands

<sup>30</sup>National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands

<sup>31</sup>Cancer Registry and Histopathology Unit, "Civile - M.P. Arezzo" Hospital, Ragusa, Italy

<sup>32</sup>Associazione Iblea per la Ricerca Epidemiologica - Onlus, Ragusa, Italy

<sup>33</sup>Division of Human Nutrition, Section of Nutrition and Epidemiology, Wageningen University, Wageningen, the Netherlands

Corresponding author: Clara Podmore, clara.podmore@mrc-epid.cam.ac.uk.

Received 4 February 2015 and accepted 10 January 2016.

This article contains Supplementary Data online at <http://care.diabetesjournals.org/lookup/suppl/doi:10.2337/dc15-0257/-/DC1>.

© 2016 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered.

Oxford, and Heidelberg. Menopausal status was defined as menopausal (postmenopausal or posthysterectomy) and nonmenopausal (pre- or perimenopausal). A blood sample was taken at varying times of day and stored frozen for future measurements (15). Follow-up data on mortality and disease status was ascertained via registries, clinical records, and other sources of clinical information (15).

#### **Type 2 Diabetes Case Ascertainment and Verification**

Incident type 2 diabetes cases were identified using multiple sources of evidence, including self-report, linkage to primary-care registers, linkage to secondary-care registers, medication use, hospital admissions, and mortality data. Cases were considered verified if confirmed by at least two independent sources. Cases in Denmark and Sweden were identified via local and national diabetes and pharmaceutical registers, and hence all ascertained cases were considered to be verified (15). Follow-up was censored at the date of diagnosis, 31 December 2007, or date of death, whichever occurred first.

#### **Laboratory Measurements**

Serum samples were used to measure the biomarkers in all centers except Umea ( $n = 1,845$ ), where only plasma samples were available and only ferritin could be measured. All measurements were done at the Stichting Huisartsen Laboratorium, Etten-Leur, the Netherlands. Cobas assays were used to measure ferritin (electrochemiluminescence immunoassay sandwich principle), iron (colorimetric assay), and transferrin (immunoturbidimetric assay) on a Roche Hitachi Modular P analyzer. The assay range for serum iron was  $0.9\text{--}179\text{ }\mu\text{mol/L}$ , that for transferrin was  $1.26\text{--}63\text{ }\mu\text{mol/L}$ , and that for ferritin was  $0.5\text{--}2000\text{ }\mu\text{g/L}$ . Results below the lower detection limit for each assay were considered missing (only two results for serum iron). TSAT was calculated as follows:  $[\text{iron } (\mu\text{mol/L}) \times 100] / [\text{transferrin } (\text{g/L}) \times 22.75]$ . Cobas assays on the same analyzer were also used to measure hs-CRP (particle-enhanced immunoturbidimetric assay), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) (ultraviolet test), and  $\gamma$ -glutamyl transferase (GGT) (enzymatic calorimetric assay). Quality control was based on the Westgard rules (17).

#### **Statistical Analysis**

Baseline characteristics of individuals in the subcohort were compared across sex-specific quartiles of ferritin distribution. Distributions of ferritin levels were compared by sex, BMI, and waist circumference categories in the subcohort. After log-transformation of variables with skewed distributions (ferritin, hs-CRP, GGT, and alcohol consumption), a multivariable regression model adjusted for age, center, and sex, and unadjusted Pearson correlation coefficients, were used to describe the relationships between each biomarker of iron metabolism and each other, and with possible confounders.

We estimated associations of differences (defined in Table 3) in ferritin, iron, and transferrin (in natural units) with the risk of type 2 diabetes using Prentice-weighted Cox regression models with age as the underlying timescale, fitted separately within each country; estimates were combined across countries using random effects meta-analysis. Prentice-weighted Cox regression is used to analyze a case-cohort study to account for the enrichment of incident cases occurring outside of the random subcohort. We used hazard ratios (HRs) as estimates of risk. We used TSAT  $\geq 45\%$  as a cutoff because this is the threshold recommended by clinical guidelines to rule out genetic causes of hyperferritinemia (18) and at which substantial levels of non-transferrin-bound iron appear (14). We fitted three different models with increasing levels of adjustment for key potential confounders, namely, age, study center, BMI, physical activity, smoking status, level of education, hs-CRP, ALT, and GGT. AST and ALT were highly correlated ( $r = 0.75$ ), and because AST is less specific for liver disease than ALT, we included only ALT in the model. We included participants who had data available for the relevant biomarker and all these potential confounders, unless stated otherwise. To compare results with pooled estimates from a recent meta-analysis (3), results were also reported for the top quintile compared with the lowest quintile of ferritin (sex-specific quintiles defined in the subcohort). Because the distribution of ferritin is substantially different among men and women in the general population, we also reported results for one sex-specific SD of ferritin. We

also presented HRs for various TSAT cut-offs and for a 5% higher level of TSAT. Adjusted and unadjusted cubic splines were generated for the association of each biomarker with type 2 diabetes in men and women. The splines were calculated between the 1st and 99th percentiles of the relevant biomarker, with knots at the 5th, 25th, 75th, and 95th percentiles and the median as the reference.

The association of ferritin with type 2 diabetes was also estimated in a restricted sample of individuals who did not present signs of common correlates of hyperferritinemia, namely, inflammation, liver disease, high alcohol consumption, and obesity ( $n = 10,958$ ). These were defined as individuals with hs-CRP  $< 10\text{ mg/L}$ , ALT and AST  $\leq 40\text{ U/L}$ , GGT  $\leq 60\text{ U/L}$  (men) and  $\leq 40\text{ U/L}$  (women) and low to moderate self-reported alcohol consumption ( $< 30\text{ g/day}$  in men and  $< 20\text{ g/day}$  in women, as suggested by the European Association for the Study of Liver [19]). The same association was also estimated after excluding individuals with ferritin levels higher than  $1,000\text{ }\mu\text{g/L}$  ( $n = 125$ ) in an attempt to exclude individuals with conditions of extreme iron overload, such as HHC.

For biomarkers that showed a significant association with type 2 diabetes in men and women,  $P$  values for interaction between the biomarker and variables related to iron metabolism were estimated by including a parameter representing the interaction between the biomarker (continuous) and the variable of interest (categorical) in Prentice-weighted Cox regression models adjusted for age, sex, and center and fitted within each country; estimates were combined using random effects meta-analysis. HRs of type 2 diabetes for each biomarker were then estimated within strata for each variable of interest. Waist circumference was categorized according to sex-specific cutoffs (20) and BMI according to the World Health Organization classification (defined in Fig. 2).

Sensitivity analyses were carried out for the association of ferritin and type 2 diabetes because it is the one where confounding is most likely; this was also adjusted for menopausal status, alcohol consumption, and red meat

consumption. Information on waist circumference and family history of type 2 diabetes was missing in 7.3% and 50.4% of the study population, respectively, mainly because it had not been assessed by certain centers. Therefore, these variables were not included as covariates in the main models, but sensitivity analyses were carried out among individuals with information on waist circumference ( $n = 23,122$ ) and family history of type 2 diabetes ( $n = 11,565$ ). All analyses were performed using Stata 13.

## RESULTS

Of all 27,779 InterAct participants (12,403 incident type 2 diabetes cases), between 23,554 (10,371 cases) and 25,113 individuals (11,052 cases) had data available for the relevant biomarkers and all the covariates for the main

models and were included in this analysis. The median (interquartile range) of ferritin in the subcohort was 144  $\mu\text{g/L}$  (80–241  $\mu\text{g/L}$ ) in men and 58  $\mu\text{g/L}$  (29–107  $\mu\text{g/L}$ ) in women. Among those in the subcohort, 8.31% of men and 4.78% of women had TSAT  $\geq 45\%$ . Summary statistics of biomarkers and baseline characteristics of participants by quartiles of ferritin in the subcohort are detailed in Tables 1 and 2. Individuals in the highest quartile of ferritin were older, consumed more alcohol, had lower levels of transferrin, and had higher levels of TSAT and liver enzymes compared with individuals in the rest of the subcohort. Leaner individuals had smaller SDs of ferritin (Supplementary Table 1). In linear regression analyses adjusting for age, sex, and center (Supplementary Table 2), ferritin was associated with each of the other iron markers and with all of

the possible confounding factors with the exception of estimated dietary iron intake, for which the relationship was weak. TSAT was strongly correlated with serum iron ( $r = 0.91$ ) and inversely correlated with hs-CRP ( $r = -0.15$ ). Estimated dietary iron intake was only weakly associated with ferritin and not with the other iron biomarkers.

HRs of type 2 diabetes for each biomarker are summarized in Table 3 and Supplementary Fig. 1; the adjusted and unadjusted associations estimated from spline regression are displayed in Fig. 1 and Supplementary Fig. 2, respectively. A 100  $\mu\text{g/L}$  higher ferritin level was associated with a higher risk (95% CI) of type 2 diabetes in men (1.07 [1.01–1.12]) and women (1.12 [1.05–1.19]), after adjustment for age, center, BMI, physical activity, smoking status, level of education, hs-CRP, ALT, and GGT.

**Table 1—Baseline characteristics by quartiles of ferritin in men in the subcohort ( $n = 5,697$ )**

	Ferritin by quartile* ( $\mu\text{g/L}$ )				<i>P</i> for difference across quartiles†	Ferritin in the overall subcohort ( $\mu\text{g/L}$ )
	Q1 (4–80)	Q2 (81–144)	Q3 (145–241)	Q4 (242–283)		
Age (years)	52.2 (8.9)	52.1 (9.1)	52.8 (9.1)	53.5 (8.3)	<0.001	52.9 (8.9)
BMI ( $\text{kg/m}^2$ )	26.2 (3.5)	26.4 (3.5)	26.6 (3.5)	27.4 (3.6)	<0.001	26.6 (3.6)
Education					0.0001	
Low	7.9	6.1	4.9	4.4		5.6
Primary	37.0	35.0	31.5	32.0		34.1
Vocational	21.4	21.4	22.7	25.3		22.8
Secondary	13.2	13.3	13.7	12.3		13.3
Higher	20.5	24.1	27.2	26.1		24.2
Physical activity					0.006	
Inactive	17.5	17.8	19.7	18.9		18.7
Moderately inactive	28.6	29.9	30.7	34.0		30.9
Moderately active	25.9	27.1	24.2	24.8		25.5
Active	28.0	25.1	25.2	22.3		24.9
Smoking					0.7	
Never	34.5	32.8	30.6	29.2		31.7
Former	32.5	32.5	37.7	42.6		36.7
Current	33.0	34.7	31.6	28.2		31.6
Family history of type 2 diabetes	12.1	16.2	12.8	19.3	<0.001	15.5
Alcohol intake (g/day)‡	10.5 (2.2–24.6)	12.3 (3.4–28.3)	15.2 (5.4–36.2)	19.3 (7.1–40.2)	<0.001	13.5 (4.0–32.4)
Dietary iron intake (mg/day)	15.3 (4.9)	15.4 (5.0)	15.2 (5.0)	15.3 (5.0)	0.9	15.3 (5.0)
Biomarkers						
TSAT (%)	26.8 (9.9)	29.7 (9.8)	30.8 (9.2)	33.8 (12.5)	<0.001	30.3 (10.7)
Iron ( $\mu\text{mol/L}$ )	17.3 (6.0)	17.8 (5.7)	18.2 (5.5)	19.2 (6.6)	<0.001	18.1 (6.0)
Transferrin (g/L)	2.9 (0.4)	2.7 (0.4)	2.6 (0.3)	2.5 (0.4)	<0.001	2.7 (0.4)
Glucose (mmol/L)	5.0 (1.6)	5.1 (1.3)	5.2 (1.5)	5.6 (1.5)	<0.001	5.2 (1.5)
HbA <sub>1c</sub> (mmol/mol)	36.4 (4.8)	36.3 (4.5)	36.4 (5.4)	36.3 (6.4)	0.97	36.4 (5.3)
hs-CRP (mg/L)‡	0.9 (0.5–1.9)	1.0 (0.5–2.1)	1.1 (0.6–2.5)	1.4 (0.7–2.9)	<0.001	1.1 (0.5–2.3)
ALT (U/L)	22.6 (10.4)	24.4 (11.8)	26.6 (13.6)	32.9 (20.0)	<0.001	26.6 (14.9)
AST (U/L)	29.3 (7.6)	30.0 (8.4)	30.9 (9.5)	35.0 (15.3)	<0.001	31.3 (10.9)
GGT (U/L)‡	24.0 (18.0–34.0)	26.0 (19.0–40.0)	29.5 (21.0–46.0)	36.0 (25.0–60.0)	<0.001	28.0 (20.0–44.0)

Data are mean (SD) or percentage. Data for skewed variables (marked with a ‡) are shown as median (interquartile range). \*Range of ferritin values by quartile are in parentheses. †ANOVA for normally distributed continuous variables, Kruskal-Wallis test for continuous variables with skewed distribution (marked with a ‡), and  $\chi^2$  test for categorical variables.

**Table 2—Baseline characteristics by quartiles of ferritin in women in the subcohort (n = 9,485)**

	Ferritin by quartile* (μg/L)				P for difference across quartiles†	Ferritin in the overall subcohort (μg/L)
	Q1 (1–29)	Q2 (30–58)	Q3 (59–107)	Q4 (108–3,017)		
Age (years)	47.2 (8.0)	50.0 (9.4)	54.1 (8.7)	57.0 (7.8)	<0.001	52.1 (9.3)
BMI (kg/m <sup>2</sup> )	25.5 (4.4)	25.4 (4.6)	25.4 (4.4)	26.4 (4.6)	<0.001	25.7 (4.5)
Education					0.002	
Low	12.4	10.2	7.3	6.3		8.9
Primary	33.1	30.5	32.6	34.2		32.8
Vocational	20.3	22.0	24.4	26.6		23.4
Secondary	15.6	16.6	18.0	17.0		16.6
Higher	18.6	20.7	17.8	15.9		18.3
Physical activity					0.0008	
Inactive	30.1	26.4	26.8	24.5		27.0
Moderately inactive	34.2	35.4	34.8	36.2		35.1
Moderately active	20.3	20.9	20.4	21.1		20.9
Active	15.4	17.3	18.0	18.2		17.1
Smoking					0.0006	
Never	59.7	54.6	54.1	56.2		56.0
Former	20.2	21.3	21.7	22.4		21.4
Current	20.1	24.1	24.3	21.4		22.6
Family history of type 2 diabetes	19.7	18.7	19.5	23.5	0.1	20.5
Alcohol intake (g/day)‡	1.7 (0–7.2)	2.7 (0.2–10.6)	3.6 (0.4–12.0)	5.1 (0.6–13.5)	<0.001	3.0 (0.2–11.1)
Estimated dietary iron intake (mg/day)	12.3 (3.6)	12.4 (3.7)	12.3 (3.6)	12.0 (3.4)	0.009	12.2 (3.6)
Biomarkers						
TSAT (%)	20.8 (10.5)	27.2 (9.7)	28.5 (9.0)	30.5 (10.3)	<0.001	26.7 (10.5)
Iron (μmol/L)	14.3 (6.7)	17.1 (6.0)	17.3 (5.4)	17.8 (5.8)	<0.001	16.6 (6.2)
Transferrin (g/L)	3.1 (0.5)	2.8 (0.4)	2.7 (0.4)	2.6 (0.4)	<0.001	2.8 (0.4)
Glucose (mmol/L)	4.7 (1.3)	4.7 (1.0)	4.8 (1.2)	5.0 (1.3)	<0.001	4.8 (1.2)
HbA <sub>1c</sub> (mmol/mol)	35.6 (4.6)	35.5 (4.3)	36.4 (4.7)	36.9 (5.8)	<0.001	36.1 (4.9)
hs-CRP (mg/L)‡	0.9 (0.4–1.9)	1.0 (0.5–2.2)	1.1 (0.6–2.5)	1.4 (0.7–3.1)	<0.001	1.1 (0.5–2.4)
ALT (U/L)	17.0 (8.7)	17.7 (9.0)	19.5 (12.4)	21.9 (15.1)	<0.001	19.0 (11.7)
AST (U/L)	25.7 (9.9)	26.1 (10.1)	27.3 (7.9)	29.1 (13.7)	<0.001	27.1 (10.7)
GGT (U/L)‡	14.0 (11.0–18.0)	16.0 (12.0–22.0)	18.0 (14.0–26.0)	20.0 (15.0–31.0)	<0.001	17.0 (13.0–24.0)

Data are mean (SD) or percentages. Data for skewed variables (marked with a ‡) are median (interquartile range). \*Range of ferritin values by quartile are in parentheses. †ANOVA for normally distributed continuous variables, Kruskal-Wallis test for continuous variables with skewed distribution (marked with a ‡), and  $\chi^2$  test for categorical variables.

HRs per sex-specific SD of ferritin were similar in men and women (Supplementary Table 3). The spline analyses showed that the strengths of the associations were weakened by the adjustment (particularly for ferritin), whereas the shapes of the associations remained generally similar. TSAT  $\geq 45\%$  versus  $<45\%$  was associated with a significantly lower risk of type 2 diabetes in women only. Using cutoffs of 50% and 55% for TSAT or estimating HRs per 5% higher level of TSAT did not substantially affect the results, but the association was no longer statistically significant in women using cutoffs of 50% or 55% (Supplementary Table 4). A higher serum iron level was not associated with type 2 diabetes. A higher transferrin level was associated with a higher risk of type 2 diabetes in men (1.11 [1.00–1.24]) and women

(1.22 [1.12–1.33]). The associations of ferritin and transferrin with type 2 diabetes were most attenuated after adjustment for BMI and ALT (data not shown). Sensitivity analyses excluding individuals who developed diabetes within the first 2 years of follow-up did not change the results (data not shown).

Restricting the analyses to individuals not presenting any sign of overt inflammation, liver disease, high alcohol consumption, or obesity moderately weakened the association of ferritin with type 2 diabetes in men to an HR (95% CI) of 1.04 (0.96–1.12), whereas the association remained similar in women, with an HR of 1.12 (1.02–1.24) per 100 μg/L higher level of ferritin, adjusted for age, center, type 2 diabetes risk factors, hs-CRP, and liver enzymes. Among individuals

with ferritin levels  $<1,000$  μg/L, the association of ferritin with type 2 diabetes was similar in men, with an HR of 1.09 (1.02–1.15), but higher in women (1.26 [1.15–1.38]).

In age- and center-adjusted analyses, associations for ferritin, TSAT, serum iron, and transferrin were stronger among women compared with men, although differences did not reach conventional levels of statistical significance for transferrin ( $P_{\text{interaction}} = 0.004$ ,  $<0.001$ , 0.01, and 0.47, respectively). There was a stronger association of ferritin with type 2 diabetes among leaner individuals (Fig. 2), with a significant interaction with waist circumference ( $P_{\text{interaction}} = 0.004$ ) and BMI ( $P_{\text{interaction}} < 0.001$ ). Transferrin showed a stronger association with type 2 diabetes among individuals at extremes of waist circumference ( $P_{\text{interaction}} = 0.034$ ). There was no interaction of either

**Table 3—HRs (95% CI) of type 2 diabetes for the higher biomarker levels stated, by sex and meta-analyzed across countries**

Biomarkers by sex	HR (95% CI)	P value	Heterogeneity I <sup>2</sup> (%)
<b>Men</b>			
Ferritin (per 100 µg/L)			
Model 1	1.18 (1.12–1.25)	<0.001	
Model 2	1.13 (1.08–1.19)	<0.001	
Model 3	1.06 (1.01–1.12)	0.021	72.20
TSAT ≥45%			
Model 1	0.99 (0.81–1.20)	0.885	
Model 2	1.06 (0.86–1.32)	0.579	
Model 3	0.90 (0.75–1.08)	0.259	0.0
Serum iron (per 5 µmol/L)			
Model 1	1.03 (0.98–1.08)	0.293	
Model 2	1.04 (0.98–1.11)	0.166	
Model 3	1.00 (0.94–1.07)	0.976	49.40
Transferrin (per 0.5 g/L)			
Model 1	1.20 (1.12–1.30)	<0.001	
Model 2	1.16 (1.05–1.29)	0.003	
Model 3	1.11 (1.00–1.23)	0.061	54.50
<b>Women</b>			
Ferritin (per 100 µg/L)			
Model 1	1.31 (1.22–1.41)	<0.001	
Model 2	1.22 (1.14–1.31)	<0.001	
Model 3	1.12 (1.05–1.19)	0.001	53.50
TSAT ≥45%			
Model 1	0.54 (0.44–0.67)	<0.001	
Model 2	0.73 (0.59–0.91)	0.004	
Model 3	0.68 (0.54–0.86)	0.002	0.0
Serum iron (per 5 µmol/L)			
Model 1	0.92 (0.89–0.95)	<0.001	
Model 2	1.02 (0.97–1.07)	0.403	
Model 3	1.00 (0.95–1.05)	0.869	38.00
Transferrin (per 0.5 g/L)			
Model 1	1.30 (1.21–1.41)	<0.001	
Model 2	1.24 (1.15–1.34)	<0.001	
Model 3	1.22 (1.12–1.33)	<0.001	55.30
<b>Men and women</b>			
Ferritin (upper vs. lower quintile)†			
Model 1*	2.46 (2.05–2.96)	<0.001	
Model 2*	1.77 (1.57–2.00)	<0.001	
Model 3*	1.36 (1.20–1.54)	<0.001	5.3

Model 1 is adjusted for age and center. Model 2 is further adjusted for BMI, physical activity, smoking status, and level of education. Model 3 is adjusted even further for hs-CRP, ALT, and GGT. \*Additional adjustment for sex. †Ferritin quintile cut points for men: ≤68, >68–117, >117–177, >177–270, and >270 µg/L; for women: ≤24, >24–45, >45–73, >73–121, and >121 µg/L.

ferritin or transferrin with menopausal status, estimated dietary iron, or alcohol consumption, and no interaction of transferrin with BMI.

Adjusting for menopausal status, alcohol consumption, red meat intake, family history of type 2 diabetes, or waist circumference did not substantially modify the association of ferritin among men or women (Supplementary Table 5).

## CONCLUSIONS

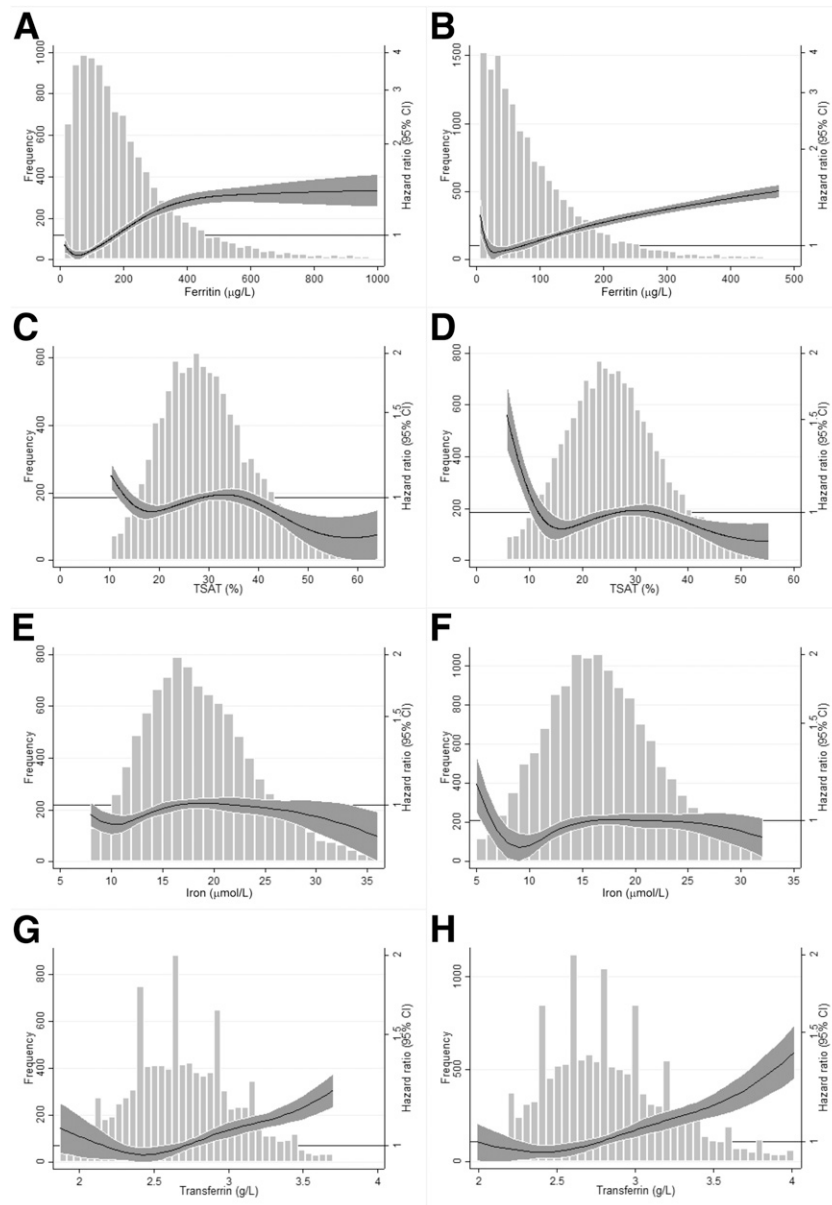
This study, which was conducted in a large European population, showed that higher ferritin and transferrin

levels were associated with an increased risk of type 2 diabetes among men and women. Even among individuals showing no signs of overt inflammation, liver disease, high alcohol consumption, or obesity, ferritin was associated with type 2 diabetes in women and to a lesser extent in men. An elevated TSAT was associated with a lower risk of type 2 diabetes in women when a cutoff of 45% was used, and serum iron was not associated with type 2 diabetes. The associations of all four iron biomarkers with type 2 diabetes were stronger among women than men. This likely

reflects physiological differences in iron metabolism and biomarker distributions between men and women, causing the relative risk of absolute biomarker differences to be greater in women.

The association of ferritin with type 2 diabetes has previously been reported, but the association from the latest meta-analysis of prospective studies was stronger and less precise than that found in this study, with an HR (95% CI) of 1.73 (1.35–2.22) for the top quintile compared with the lower quintile in the meta-analysis (3). This difference may be explained by the much larger number of cases in this study (11,052 versus 3,391) and the lack of adjustment for liver enzymes in many of the studies included in the meta-analysis. The stronger association among women than men using natural units was no longer apparent when using standardized units, suggesting that the stronger association is a reflection of the different distributions of ferritin among men and women in the population. Contrary to suggestions from previous studies (21,22), we did not observe a threshold effect of ferritin with incident type 2 diabetes in the InterAct study, but rather a linear association, with an increased risk even at levels of ferritin considered within the reference range. To our knowledge, we demonstrated for the first time that ferritin showed a relatively stronger association among leaner individuals. This may be because leaner individuals have a lower absolute risk of type 2 diabetes and that the SD of ferritin is smaller among leaner individuals, and hence the relative risk of ferritin is larger in leaner than in overweight or obese individuals. The association of higher transferrin with type 2 diabetes was previously reported in a small prospective study, which also showed the absence of an association of serum iron with type 2 diabetes (23).

Nevertheless, our study found a more complex relationship between TSAT and diabetes. Results from existing prospective studies of TSAT with type 2 diabetes are conflicting. A study using data from the National Health and Nutrition Examination Survey did not find any association between TSAT and type 2 diabetes using different cutoffs for TSAT (24). By contrast, a meta-analysis of three Danish studies found that TSAT ≥50%



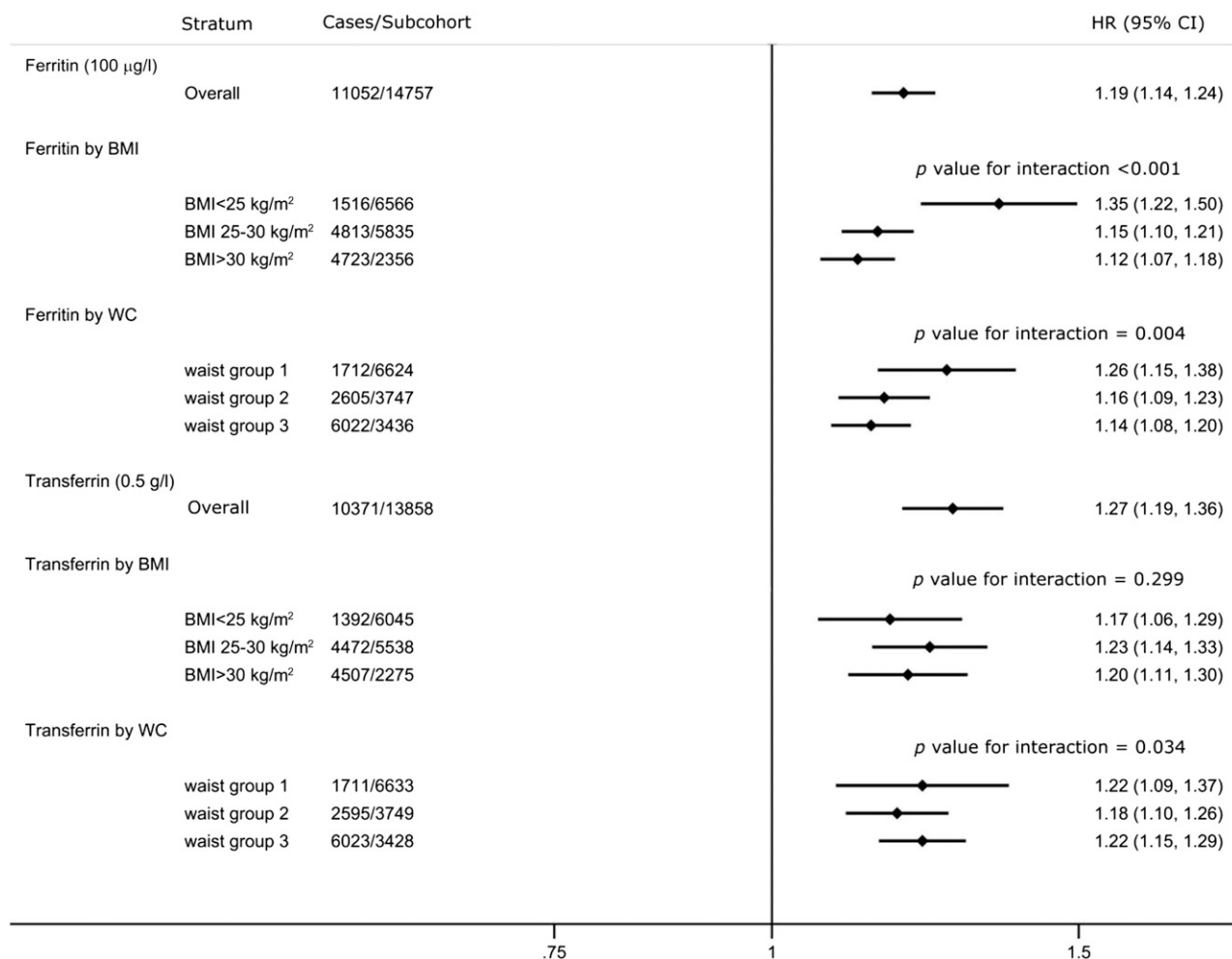
**Figure 1**—Adjusted HRs for type 2 diabetes by ferritin, TSAT, serum iron and transferrin levels in men and women. Ferritin in men (A) and women (B); TSAT in men (C) and women (D); serum iron in men (E) and women (F); and transferrin in men (G) and women (H). Adjusted for age, BMI, physical activity, smoking status, level of education, hs-CRP, ALT, and GGT (A–F and H) and age, BMI, hs-CRP, ALT, and GGT only (G) because otherwise the spline failed to converge. The histograms represent the distribution of the biomarkers in the given population.

was associated with a higher risk of type 2 diabetes (25). However, these were relatively small studies, with fewer than 1,500 cases in each. This is, to our knowledge, the first prospective study to show that elevated TSAT is associated with a lower risk of type 2 diabetes, which was statistically significant only in women. Recent cross-sectional studies have shown a similar association of high ferritin and low TSAT among individuals with “prediabetes” (26–28). TSAT is a useful biomarker of iron

metabolism in addition to ferritin (26) because TSAT levels are less affected by inflammation than ferritin (29) and are thought to reflect levels of non-transferrin-bound iron (13,14). In patients with HHC, which is characterized by high iron absorption, TSAT is elevated first, followed by ferritin once iron accumulates in tissues (1). Non-transferrin-bound iron is thought to be an important source of organ iron deposition and toxicity because it is avidly taken up by tissues, independent of the transferrin

receptor (14), and levels have been shown to be higher in patients with type 2 diabetes compared with controls (30). However, the direction of association between TSAT and type 2 diabetes observed in this study does not support a simple association between increased iron absorption or higher non-transferrin-bound iron and type 2 diabetes. This may be because not all cases of iron overload are characterized by elevated TSAT. For example, the insulin resistance–associated hepatic iron overload syndrome is characterized by mild to moderate hepatic iron overload on liver biopsy, generally with elevated ferritin but normal TSAT (31–33). Alternatively, a higher TSAT could reflect more successful scavenging of non-transferrin-bound iron and therefore be protective against type 2 diabetes. Finally, because TSAT is inversely associated with inflammation, negative confounding by inflammation may mask an association of TSAT with type 2 diabetes.

High levels of ferritin and transferrin are markers of high and low iron stores, respectively, and were strongly inversely correlated. However, they were both positively associated with type 2 diabetes in this study. Participants with low ferritin levels had a lower risk of developing type 2 diabetes compared with the median, suggesting that low iron stores per se are not associated with a higher risk of type 2 diabetes. While cross-talk between iron and insulin resistance is likely, the initiating factor of the vicious circle remains unclear (34). Cross-sectional studies showed that ferritin was correlated with 2-h glucose concentration and inversely correlated with insulin sensitivity in individuals without type 2 diabetes (8), as well as inversely with adiponectin (35,36). A recent study showed that ferritin and transferrin were prospectively associated with indices of hepatic, muscular, and adipocyte insulin resistance (37). Some experimental studies report upregulation of transferrin expression by insulin in human hepatocytes (38,39), whereas others suggest an antagonist effect of transferrin on insulin action, leading to insulin resistance (40). We suggest that the association of both ferritin and transferrin with incident type 2 diabetes could be explained,



**Figure 2**—HRs of type 2 diabetes per 100 µg/L of ferritin and 0.5 g/L of transferrin in men and women by strata adjusting for age, sex, and center, and meta-analyzed across countries. Waist circumference (WC) in men: group 1, <94 cm; group 2, 94–102 cm; group 3, ≥102 cm; in women: group 1, <80 cm; group 2, 80–88 cm; group 3, ≥88 cm.

at least in part, by insulin resistance. This is supported by the fact that in this study the strength of the associations of ferritin and transferrin with type 2 diabetes was most strongly attenuated after adjustment for BMI and ALT, which are both associated with insulin resistance (41,42).

Taken together, these observed associations of TSAT and transferrin with type 2 diabetes do not support the clear role of iron in the pathogenesis of type 2 diabetes that might have been suggested by the association of ferritin. Case series in the 20th century reported a relatively high prevalence of diabetes among patients with HHC, which formed an important basis for the hypothesized role of iron in the pathogenesis of type 2 diabetes. However, the Hemochromatosis and Iron Overload Screening Study found the sex- and age-adjusted prevalence of

self-reported diabetes similar in C282Y homozygotes and in participants without *HFE* C282Y and H63D mutations (43–46). Also, genetic studies to date show that C282Y is not associated with type 2 diabetes, whereas H63D is modestly associated (47,48). HHC was historically defined in the 19th century as the co-occurrence of cirrhosis, diabetes, and skin pigmentation, and this triad became the sine qua non of HHC until the end of the 20th century. Because diabetes was part of the triad used to define HHC, clinicians would look for type 2 diabetes in people they suspected of having HHC. This creates an ascertainment bias, which is less likely to occur now that genetic testing is the gold standard for the diagnosis of HHC. An alternative explanation for the associations observed in this study is that iron overload disorders caused by different mechanisms show different

associations with type 2 diabetes, but that these differences may not be captured by the use of biomarkers. However, the use of more invasive measures of iron stores necessary to distinguish these disorders is unlikely to be feasible on a large scale.

This is, to our knowledge, the first prospective study to comprehensively report the association of four commonly used clinical measures of iron stores with type 2 diabetes. It is limited because we had a single measure of TSAT for each individual and because most samples were collected during a non-fasting state, which may have affected the dichotomous categorization of TSAT. However, this applied to all participants irrespective of their diabetes status, and the consequence would be nondifferential error, which would underestimate the strength of the association between TSAT and type 2

diabetes. This could contribute to the lack of observed association in men but does not explain the association in women. Also, we were unable to exclude participants with clinically diagnosed HHC. However, although *HFE* mutations are common, the clinical penetrance of the disease is extremely low (49); therefore, this is unlikely to have substantially affected our results. As with all observational studies, we cannot exclude reverse causality or residual confounding as potential explanations for our findings. However, there was no relationship between the iron biomarkers and HbA<sub>1c</sub> at baseline in the subcohort, and sensitivity analyses excluding individuals who developed diabetes within the first 2 years of follow-up did not change the results. These observations lessen the likelihood of reverse causality.

In conclusion, the observed pattern of association of these biomarkers of iron metabolism with type 2 diabetes suggests a more complex relationship than simply high iron stores being a risk factor for type 2 diabetes. It remains to be clarified whether the associations of higher ferritin and transferrin with type 2 diabetes are due to a causal role of iron in the pathogenesis of type 2 diabetes, or whether it simply reflects the underlying progression of insulin resistance. The genetics of iron metabolism in general and of different disorders of iron metabolism in particular, based on their mechanisms, may be useful in addressing these questions, which are difficult to answer using traditional observational designs.

**Acknowledgments.** The authors thank all EPIC participants and staff for their contribution to the study. The authors thank Nicola Kerrison (MRC Epidemiology Unit, University of Cambridge, Cambridge, U.K.) for managing the data for the InterAct Project. The authors thank Dr. Felix Day (MRC Epidemiology Unit, University of Cambridge, Cambridge, U.K.) for assistance with the figures. The authors also particularly thank staff from the Laboratory, Field Epidemiology and Data Teams of the MRC Epidemiology Unit in Cambridge, U.K., for carrying out sample preparation, providing DNA and DNA quality control, genotyping, and data-handling work. The authors specifically thank S. Dawson and V. Kaimakis for coordinating sample provision for biomarker measurements, and M. Sims for writing the technical laboratory specification for the intermediate pathway biomarker measurements and for overseeing the laboratory work. The authors thank the SHL-Groep, Etten-Leur,

Netherlands, where the biomarkers were measured.

**Funding.** Funding for the InterAct Project was provided by the EU FP6 Programme (grant LSHM-CT-2006\_037197). Biomarker measurements in the EPIC-InterAct subcohort were partially funded by a grant from the U.K. Medical Research Council and the British Heart Foundation (EPIC-Heart: G0800270). C.P. is funded by the Wellcome Trust (grant 097451/Z/11/Z). P.W.F. has received funding from Swedish Research Council, Swedish Diabetes Association, and Swedish Heart-Lung Foundation. V.K. and T.K. have received funding from German Cancer Aid, German Cancer Research Center (DKFZ) and German Federal Ministry of Education and Research (BMBF). P.J. has received funding from The Health Research Funds (grant RD12/0036/0018) and AGAUR, Generalitat de Catalunya (exp. 2014 SGR 726). T.J.K. has received funding from Cancer Research UK. P.M.N. has received funding from Swedish Research Council. K.O. has received funding from Danish Cancer Society. J.R.Q. has received funding from Asturias Regional Government. O.R. has received funding from the Västerbotten County Council. I.S., A.M.W.S., D.L.v.d.A., and Y.T.v.d.S. have received funding from Dutch Ministry of Public Health, Welfare and Sports (VWS), Netherlands Cancer Registry (NKR), LK Research Funds, Dutch Prevention Funds, Dutch ZON (Zorg Onderzoek Nederland), World Cancer Research Fund (WCRF), and Statistics Netherlands. Verification of the Dutch diabetes cases was additionally funded by NL Agency (grant IGE05012) and an Incentive Grant from the Board of the UMC Utrecht to Y.T.v.d.S. A.T. has received funding from Danish Cancer Society; R.T. from AIRE-ONLUS Ragusa and Sicilian Regional Government; and E.R. from Imperial College Biomedical Research Centre.

**Duality of Interest.** P.W.F. has received funding from Novo Nordisk. No other potential conflicts of interest relevant to this article were reported.

**Author Contributions.** C.P. analyzed the data and drafted the manuscript. C.P., K.M., M.B.S., R.A.S., A.R., A.S.B., E.D.A., J.D., L.A., A.B., H.B., F.C.-C., A.J.C., C.C.D., G.F., P.W.F., D.G., S.G., M.J.G., G.G., P.J., V.K., T.J.K., T.K., A.M., P.M.N., A.O., K.O., D.P., J.R.Q., O.R., C.S., E.S.-C., N.S., I.S., A.M.W.S., A.T., R.T., D.L.v.d.A., Y.T.v.d.S., E.J.M.F., N.G.F., S.J.S., E.R., C.L., and N.J.W. conceived and designed the study, interpreted data, and revised the article. N.J.W. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

## References

- Pietrangelo A. Hereditary hemochromatosis: pathogenesis, diagnosis, and treatment. *Gastroenterology* 2010;139:393–408, 408.e1–2
- Noetzi LJ, Mittelman SD, Watanabe RM, Coates TD, Wood JC. Pancreatic iron and glucose dysregulation in thalassemia major. *Am J Hematol* 2012;87:155–160
- Kunutsor SK, Apekey TA, Walley J, Kain K. Ferritin levels and risk of type 2 diabetes mellitus: an updated systematic review and meta-analysis of prospective evidence. *Diabetes Metab Res Rev* 2013;29:308–318

- Orban E, Schwab S, Thorand B, Huth C. Association of iron indices and type 2 diabetes: a meta-analysis of observational studies. *Diabetes Metab Res Rev* 2014;30:372–394
- Walters GO, Miller FM, Worwood M. Serum ferritin concentration and iron stores in normal subjects. *J Clin Pathol* 1973;26:770–772
- Jacobs A, Miller F, Worwood M, Beamish MR, Wardrop CA. Ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. *BMJ* 1972;4:206–208
- Beutler E, Felitti V, Ho NJ, Gelbart T. Relationship of body iron stores to levels of serum ferritin, serum iron, unsaturated iron binding capacity and transferrin saturation in patients with iron storage disease. *Acta Haematol* 2002;107:145–149
- Haap M, Fritsche A, Mensing HJ, Häring HU, Stumvoll M. Association of high serum ferritin concentration with glucose intolerance and insulin resistance in healthy people. *Ann Intern Med* 2003;139:869–871
- Leggett BA, Brown NN, Bryant SJ, Duplock L, Powell LW, Halliday JW. Factors affecting the concentrations of ferritin in serum in a healthy Australian population. *Clin Chem* 1990;36:1350–1355
- Byrne CD. Dorothy Hodgkin Lecture 2012: non-alcoholic fatty liver disease, insulin resistance and ectopic fat: a new problem in diabetes management. *Diabet Med* 2012;29:1098–1107
- Lontchi-Yimagou E, Sobngwi E, Matsha TE, Kengne AP. Diabetes mellitus and inflammation. *Curr Diab Rep* 2013;13:435–444
- Worwood M. The laboratory assessment of iron status—an update. *Clin Chim Acta* 1997;259:3–23
- Fleming RE, Ponka P. Iron overload in human disease. *N Engl J Med* 2012;366:348–359
- Brissot P, Ropert M, Le Lan C, Loréal O. Non-transferrin bound iron: a key role in iron overload and iron toxicity. *Biochim Biophys Acta* 2012;1820:403–410
- Langenberg C, Sharp S, Forouhi NG, et al.; InterAct Consortium. Design and cohort description of the InterAct Project: an examination of the interaction of genetic and lifestyle factors on the incidence of type 2 diabetes in the EPIC Study. *Diabetologia* 2011;54:2272–2282
- Peters T, Brage S, Westgate K, et al.; InterAct Consortium. Validity of a short questionnaire to assess physical activity in 10 European countries. *Eur J Epidemiol* 2012;27:15–25
- Westgard rules [Internet], 2009. Madison, WI, Westgard QC. Available from <http://www.westgard.com/mltirule.htm>. Accessed 25 January 2016
- European Association for the Study of the Liver. EASL clinical practice guidelines for HFE hemochromatosis. *J Hepatol* 2010;53:3–22
- Ratziu V, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol* 2010;53:372–384
- Lean ME, Han TS, Morrison CE. Waist circumference as a measure for indicating need for weight management. *BMJ* 1995;311:158–161
- Jung CH, Lee MJ, Hwang JY, et al. Elevated serum ferritin level is associated with the incident type 2 diabetes in healthy Korean men: a 4

year longitudinal study. *PLoS One* 2013;8:e75250

22. Lee B-K, Kim Y, Kim Y-I. 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* 2011;60:1416–1424
23. Fumeron F, Péan F, Driss F, et al.; Insulin Resistance Syndrome (DESIR) Study Group. 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* 2006;29:2090–2094
24. Mainous AG 3rd, King DE, Pearson WS, Garr DR. Is an elevated serum transferrin saturation associated with the development of diabetes? *J Fam Pract* 2002;51:933–936
25. Ellervik C, Mandrup-Poulsen T, Andersen HU, et al. Elevated transferrin saturation and risk of diabetes: three population-based studies. *Diabetes Care* 2011;34:2256–2258
26. Cheung CL, Cheung TT, Lam KS, Cheung BM. High ferritin and low transferrin saturation are associated with pre-diabetes among a national representative sample of U.S. adults. *Clin Nutr* 2013;32:1055–1060
27. Park RJ, Moon JD. Low transferrin saturation is associated with impaired fasting glucose and insulin resistance in the South Korean adults: the 2010 Korean National Health and Nutrition Examination Survey. *Diabet Med* 2015;32:673–678
28. Huth C, Beuerle S, Zierer A, et al. Biomarkers of iron metabolism are independently associated with impaired glucose metabolism and type 2 diabetes: the KORA F4 study. *Eur J Endocrinol* 2015;173:643–653
29. Szöke D, Panteghini M. Diagnostic value of transferrin. *Clin Chim Acta* 2012;413:1184–1189
30. Lee D-HD-H, Liu DY, Jacobs DR Jr, et al. Common presence of non-transferrin-bound iron among patients with type 2 diabetes. *Diabetes Care* 2006;29:1090–1095
31. Moirand R, Mortaji AM, Loréal O, Paillard F, Brissot P, Deugnier Y. A new syndrome of liver iron overload with normal transferrin saturation. *Lancet* 1997;349:95–97
32. Mendler MH, Turlin B, Moirand R, et al. Insulin resistance-associated hepatic iron overload. *Gastroenterology* 1999;117:1155–1163
33. Turlin B, Mendler MH, Moirand R, Guyader D, Guillygomarc'h A, Deugnier Y. Histologic features of the liver in insulin resistance-associated iron overload. A study of 139 patients. *Am J Clin Pathol* 2001;116:263–270
34. Fernández-Real JM, López-Bermejo A, Ricart W. Cross-talk between iron metabolism and diabetes. *Diabetes* 2002;51:2348–2354
35. Forouhi NG, Harding AH, Allison M, et al. Elevated serum ferritin levels predict new-onset type 2 diabetes: results from the EPIC-Norfolk prospective study. *Diabetologia* 2007;50:949–956
36. Gabrielsen JS, Gao Y, Simcox JA, et al. Adipocyte iron regulates adiponectin and insulin sensitivity. *J Clin Invest* 2012;122:3529–3540
37. Wlazlo N, van Greevenbroek MM, Ferreira I, et al. Iron metabolism is prospectively associated with insulin resistance and glucose intolerance over a 7-year follow-up period: the CODAM study. *Acta Diabetol* 2015;52:337–348
38. O'Riordain MG, Ross JA, Fearon KC, et al. Insulin and counterregulatory hormones influence acute-phase protein production in human hepatocytes. *Am J Physiol* 1995;269:E323–E330
39. Tanner LI, Lienhard GE. Insulin elicits a redistribution of transferrin receptors in 3T3-L1 adipocytes through an increase in the rate constant for receptor externalization. *J Biol Chem* 1987;262:8975–8980
40. Vargas L, Kawada ME, Bazaes S, et al. Insulin antagonism: a novel role for human serum transferrin. *Horm Metab Res* 1998;30:113–117.
41. Hanley AJ, Williams K, Festa A, et al.; Insulin Resistance Atherosclerosis Study. Elevations in markers of liver injury and risk of type 2 diabetes: the insulin resistance atherosclerosis study. *Diabetes* 2004;53:2623–2632
42. Nolan CJ, Damm P, Prentki M. Type 2 diabetes across generations: from pathophysiology to prevention and management. *Lancet* 2011;378:169–181
43. Adams PC, McLaren CE, Speechley M, McLaren GD, Barton JC, Eckfeldt JH. HFE mutations in Caucasian participants of the Hemochromatosis and Iron Overload Screening study with serum ferritin level <1000 µg/L. *Can J Gastroenterol* 2013;27:390–392
44. Adams PC, Reboussin DM, Barton JC, et al.; Hemochromatosis and Iron Overload Screening (HEIRS) Study Research Investigators. Hemochromatosis and iron-overload screening in a racially diverse population. *N Engl J Med* 2005;352:1769–1778
45. Allen KJ, Gurrin LC, Constantine CC, et al. Iron-overload-related disease in HFE hereditary hemochromatosis. *N Engl J Med* 2008;358:221–230
46. Beutler E, Felitti VJ, Koziol JA, Ho NJ, Gelbart T. Penetrance of 845G→A (C282Y) HFE hereditary haemochromatosis mutation in the USA. *Lancet* 2002;359:211–218
47. Rong Y, Bao W, Rong S, et al. Hemochromatosis gene (HFE) polymorphisms and risk of type 2 diabetes mellitus: a meta-analysis. *Am J Epidemiol* 2012;176:461–472
48. Zhang D, Jiang X, Wu Y, Jiang W, Pang Z. Re: “hemochromatosis gene (HFE) polymorphisms and risk of type 2 diabetes mellitus: a meta-analysis”. *Am J Epidemiol* 2013;177:372–373
49. Bacon BR, Britton RS. Clinical penetrance of hereditary hemochromatosis. *N Engl J Med* 2008;358:291–292