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RESEARCH ARTICLE

Serum ferritin and risk for new-onset heart failure and cardiovascular events in the community

IJsbrand T. Klip[1], Adriaan A. Voors[1], Dorine W. Swinkels[2], Stephan J.L. Bakker[3], Jenny E. Kootstra-Ros[4], Carolyn S. Lam[5], Pim van der Harst[1], Dirk J. van Veldhuisen[1], and Peter van der Meer[1]*

[1]Department of Cardiology, University Medical Center Groningen, Groningen, The Netherlands; [2]Department of Laboratory Medicine, Radboud University Medical Center, Nijmegen, The Netherlands; [3]Department of Nephrology, University Medical Center Groningen, Groningen, The Netherlands; [4]Department of Laboratory Medicine, University Medical Center Groningen, Groningen, The Netherlands; and [5]Yong Loo Lin School of Medicine, National University of Singapore, Singapore

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Aims Heart failure (HF) is a common manifestation of patients with primary and secondary causes of iron overload, whereas in patients with established HF iron deficiency impairs outcome. Whether iron stores, either depleted or in overload, amplify the risk for new-onset HF among healthy individuals is unknown. The present study aimed to assess whether markers of iron status or the iron-regulatory hormone hepcidin are associated with new-onset HF or cardiovascular (CV) events in the general population.

Methods and results In 6386 subjects from the Prevention of REnal and Vascular ENd-stage Disease (PREVEND) trial, a prospective, community-based, cohort study, markers of iron status and the iron-regulatory hormone hepcidin were measured. Mean age was 53.1 ± 12.0 years, and 50.7% of the cohort was female. During a median follow-up of 8.3 (interquartile range 7.8–8.9) years, 199 subjects (3.1%) were newly diagnosed with HF, 456 (7.1%) experienced a CV event, and 356 (5.6%) died from all causes. A higher annual HF incidence per ferritin quartile was observed in women ($P < 0.001$), but not in men (P for interaction 0.032). Multivariable analyses demonstrated ferritin levels to remain independently predictive for new-onset HF in women only ($P = 0.024$). This association persisted within strata defined by markers of the metabolic syndrome, markers of inflammation, or other markers of iron homeostasis, including hepcidin. No association between ferritin or hepcidin and incident CV events or all-cause mortality was observed in either sex.

Conclusions Increased serum ferritin levels independently amplify the risk for new-onset HF in women in the community.

Keywords Iron • Hepcidin • Heart failure • Cardiovascular disease • Epidemiology

Introduction

Iron is an essential micronutrient for a wide range of metabolic and biological processes. Systemic iron homeostasis is orchestrated by hepcidin, a liver-derived peptide that has emerged as the key circulating regulator of iron absorption and tissue distribution.[1] In response to low body iron stores, decreased erythropoiesis, and hypoxia, hepcidin levels decrease, allowing efficient iron mobilization. In contrast, inflammation and increased body iron stores cause excessive hepcidin production, which subsequently inhibits intestinal iron absorption and iron release from reticuloendothelial macrophages.[1]

In patients with primary and secondary causes of iron overload, cardiomyopathy is a common manifestation.[2] In contrast, severe iron deficiency has also been associated with abnormalities in systolic and diastolic cardiac function, suggesting a U-shaped relationship between body iron stores and the process leading to heart failure (HF).[3,4] Prior epidemiological studies have proposed an association between increasing body iron stores (mostly expressed as serum ferritin levels) and risk for cardiovascular (CV) disease in the

*Corresponding author. Hanzeplein 1, PO Box 30.001, 9700 RB Groningen, The Netherlands. Tel: +31 503612355, Fax: +31 503611347, Email: y.t.klip@umcg.nl

general population, whereas others have found conflicting results.[5] Likewise, the role of hepcidin as a risk modifier in CV disease is also controversial.[6,7]

Whether iron stores, either depleted or in overload, or its regulator hepcidin, predict the risk of new-onset HF among healthy individuals is unknown. Therefore, we aimed to determine whether markers of iron homeostasis antedate the development of HF and CV outcome. For these purposes, we used data obtained in the Prevention of REnal and Vascular ENd-stage Disease (PREVEND) study, a large prospective, well-characterized, contemporary observational cohort with a long-term follow-up.

Methods

This study was performed using data of subjects participating in the PREVEND study. Details of the study protocol have been published elsewhere (www.prevend.org).[8] In brief, from 1997 to 1998, all inhabitants of the city of Groningen, The Netherlands, aged 28–75 years, were sent a questionnaire on demographics, disease history, smoking habits, and use of medication, and a vial to collect an early morning urinary sample ($n = 85\,421$). Of these subjects, 40 856 responded (47.8%). After exclusion of subjects with type 1 diabetes mellitus (defined as the use of insulin) and pregnant women, subjects with a urinary albumin excretion (UAE) ≥10 mg/L ($n = 6000$) and a randomly selected control group with an UAE <10 mg/L ($n = 2592$) completed the protocol and formed the baseline PREVEND cohort ($n = 8592$). For the current analyses, we used data from the second survey, which took place between 2001 and 2003 ($n = 6894$), as hepcidin and iron measurements were only available from this time period. We excluded 508 subjects due to missing ferritin or hepcidin values, and missing follow-up data, leaving 6386 subjects for the current analysis. The PREVEND study was approved by the institutional medical ethics committee and conducted in accordance with the Declaration of Helsinki. All subjects provided written informed consent.

Definitions and calculations

Blood pressure was calculated as the mean of the measurements of the two study visits of the second survey. Hypertension was defined as systolic blood pressure >140 mmHg, diastolic blood pressure >90 mmHg, or self-reported use of antihypertensive medication. Body mass index (BMI) was calculated as the ratio of weight and height squared (kg/m^2), and obesity was defined as a BMI >30 kg/m^2. Hypercholesterolaemia was defined as total serum cholesterol >6.5 mmol/L (251 mg/dL), a serum cholesterol ≥5.0 mmol/L (193 mg/dL) when a history of myocardial infarction (MI) was present, or when lipid-lowering medication was used. Type 2 diabetes mellitus was defined as a fasting glucose level of ≥7.0 mmol/L (126 mg/dL), a non-fasting glucose level of ≥11.1 mmol/L (200 mg/dL), or the use of antidiabetic drugs. Smoking was defined as current smoking or smoking cessation within the previous year. Urinary albumin excretion was calculated as the average UAE in the two consecutive 24-h urine collections. The estimated glomerular filtration rate (eGFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation.[9] History of MI or stroke was defined as a self-reported condition requiring hospitalization for at least 3 days. Anaemia was defined according to the World Health Organization (WHO) criteria (haemoglobin <13 g/dL for men and <12 g/dL for women).[10] Standard 12-lead ECGs were recorded using the computer Modular ECG Analysis System.[11] The presence of a left bundle branch block (LBBB) was defined as a QRS duration on ECG >120 ms. Left ventricular hypertrophy (LVH) was defined using the Cornell criteria: RaVL + SV_3 (with 6 mm added in women) multiplied by the QRS duration. A threshold of 2440 mm/ms was used to identify LVH.[12]

Analytical methods

Fasting blood samples were obtained in the morning from all participants from 2001 to 2003. Aliquots of these samples were stored immediately at −80 °C until further analysis. Haemoglobin (g/dL) was measured using a Coulter Counter STKS sum (Coulter Corporation, Miami, FL, USA). Serum iron (μmol/L), ferritin (μg/L), and transferrin (g/L) were measured using a colorimetric assay, immunoassay, and immunoturbidimetric assay, respectively (Roche Diagnostics, Mannheim, Germany). The total iron binding capacity (TIBC) was calculated by multiplying transferrin by 25.2. Subsequently, transferrin saturation [TSAT (%)] was computed by dividing serum iron by TIBC and multiplying by 100. Serum hepcidin concentrations were measured with a competitive enzyme-linked immunosorbent assay (ELISA), as described elsewhere.[13] The between-plates and interassay coefficients of variation were 8.6% and 16.2%, respectively. Hepcidin concentrations are expressed in nanomoles per litre (nmol/L). Concentrations of total cholesterol, plasma glucose, and serum creatinine were measured using standard methods. Urinary albumin concentration was determined by nephelometry, with a threshold of 2.3 mg/L and intra- and interassay coefficients of variation of 2.2% and 2.6%, respectively (BN II, Dade Behring Diagnostica, Marburg, Germany). High-sensitivity C-reactive protein (hs-CRP) was also determined using nephelometry with a threshold of 0.175 mg/L and intra- and interassay coefficients of <4.4% and 5.7%, respectively.

Definition of cardiovascular events and new-onset heart failure

Follow-up for the present study was defined as the time between the first follow-up visit to the outpatient department and the date of a CV event, new-onset HF, death, or 1 January 2011. Subjects were censored on the date they moved to an unknown destination or at the last date of follow-up (1 January 2011), whichever date came first. Information on dates and causes of death for every participant was obtained from Statistics Netherlands and coded according to the 10th revision of the International Classification of Diseases (ICD-10).[14]

The combined incidence of CV morbidity and mortality after the second survey was used. Information on hospitalization for CV morbidity was obtained from PRISMANT, the Dutch national registry of hospital discharge diagnoses, which has proven validity, with 84% of the primary diagnoses and 87% of the secondary diagnoses matching those recorded in the patients' charts.[15] In the present study, CV events were defined as follows: acute MI (ICD code 410), acute and subacute ischaemic heart disease (411), subarachnoid haemorrhage (430), intracerebral haemorrhage (431), other intracranial haemorrhage (432), occlusion or stenosis of the pre-cerebral (433) or cerebral arteries (434), coronary artery bypass grafting or percutaneous transluminal coronary angioplasty, and other vascular interventions such as percutaneous transluminal angioplasty or bypass grafting of the aorta and peripheral vessels.

Participants with a new diagnosis of HF were identified using criteria described in the European HF guidelines, and an endpoint adjudication committee ascertained the diagnosis of HF, as described elsewhere.[16] Additionally, HF was classified as HF with a reduced (HFrEF) or

preserved ejection fraction (HFpEF) based on the LVEF at the time of diagnosis. To acknowledge the most recent trends in cut-off for HFrEF and HFpEF in accordance with the most recent HF guidelines, we set the cut-off for HFpEF at ≥50%.[17] In all subjects developing new-onset HF in the present study, data on LVEF were available.

Statistical analyses

Baseline continuous variables are expressed as means with standard deviation (SD) when normally distributed, as medians with interquartile range (IQR) when distribution is skewed, or as numbers and percentages when categorical. Intergroup differences were tested using trend analyses. For further analyses, skewed variables were transformed to a log2 scale to achieve a normal distribution.

By design, the PREVEND study overselected subjects with an elevated UAE (>10 mg/L). To overcome this overselection of subjects with UAE, a statistical weighted method was applied in our regression analyses, allowing our conclusions to be generalized to the general population.[18,19] The association of ferritin with baseline variables was examined by bootstrapping the linear regression model 1000 times. Variables selected >700 times were assumed to be accurate and included in the multivariate model.

Overall and sex-stratified adjusted annual incidence rates of new-onset HF, CV events, and all-cause mortality were assessed assuming a Poisson distribution. The test for trend was calculated using the Mantel–Haenszel method. To preserve ferritin (and hepcidin) as continuous variables, multivariable fractional polynomials were employed to determine the best fitting functional form of both markers for all outcome parameters. To account for death as a competing risk for new-onset HF, a subject's follow-up time was censored at the time of death whenever death occurred before the onset of HF. Hazard ratios are then interpreted as the covariate effects on the cause-specific (sub)hazard function of the transition from HF-free to new-onset HF in a competing risks model with new-onset HF and all-cause mortality as the two absorbing states.[20] Crude analyses were consecutively adjusted for (i) age and sex; (ii) a multivariate model consisting of the following established HF and CV risk factors: age, sex, BMI, current smoking, diabetes, hypertension, hypercholesterolaemia, history of MI or stroke, LVH, renal function, levels of haemoglobin and erythropoietin, UAE, and presence of inflammation (hs-CRP >5 mg/L); and (iii) time-varying development of CV events. Similar analyses were done for incident CV events and all-cause mortality. All reported probability values are two-tailed, and a value of $P < 0.05$ was used as the nominal level of statistical significance. Models and analyses were performed using STATA software version 13.0 (StataCorp LP, College Station, TX, USA).

Results

Baseline characteristics of the 6386 subjects, stratified per ferritin quartiles, are displayed in *Table 1*. Mean age was 53.1 ± 12.0 years, and 50.7% of the cohort was female. Median ferritin concentration was 97 (48–173) μg/L and was higher in men (142 μg/L) compared with women (61 μg/L; $P < 0.001$). Iron deficiency, defined by ferritin levels <30 μg/L, was present in 928 subjects (14.5%) and more frequently observed in women (24.6% vs. 4.2% in men; $P < 0.001$). Although post-menopausal women had significantly higher ferritin levels in relation to their pre-menopausal counterparts (94 μg/L vs. 36 μg/L; $P < 0.001$), iron deficiency was still more frequently seen when compared with men (8.7% vs. 4.2% in men; $P < 0.001$).

Per increasing ferritin quartile, individuals were generally older, more often male, and more likely to have CV risk factors (e.g. diabetes or hypertension). Additionally, higher concentrations of haemoglobin, hs-CRP, and hepcidin, and higher TSAT were observed, whereas renal function worsened and erythropoieitin concentrations declined. Baseline ferritin quartiles were additionally stratified by sex (Supplementary material online, *Table S1*). Similar results were detected for both sexes, with the exception of prior MI and the presence of either LBBB or LVH, which were more frequently observed in women.

Clinical associates of ferritin levels

In both sexes, serum ferritin remained positively correlated with haemoglobin and mean corpuscular volume, other markers of iron homeostasis, and glucose concentrations. Ferritin was strongly related to CV risk factors (BMI and cholesterol) in men, whereas inflammation and decreasing erythropoietin levels were associated with ferritin levels in women (*Table 2*). In a bootstrapped model for ferritin (per doubling), these variables were highly selected. The presence of LVH (a strong HF risk factor) or LBBB was associated with ferritin levels in women (but not in men) in univariable regression only (Supplementary material online, *Table S2*). The multivariable model in women had a better model fit compared with men (*Table 2*). Similar CV risk factors related to ferritin levels in men and women without prior CV disease, and the adjusted R^2 was comparable (Supplementary material online, *Table S3*).

Markers of iron homeostasis and new-onset heart failure

After a median follow-up of 8.3 (7.8–8.9) years, 199 individuals (3.1%) were diagnosed with new-onset HF, 456 (7.1%) experienced a CV event, and 356 (5.6%) died from all causes. Compared with other markers of iron status, ferritin had the best accuracy in predicting incident HF and other events (Supplementary material online, *Figure S1*). The adjusted annual incidence of new-onset HF rose per increasing ferritin quartile (*Figure 1*). This trend only appeared to be significant in women ($P < 0.001$). Crude and multivariable fractional polynomials revealed the association between ferritin levels and the development of new-onset HF to be best described by a linear function. In subsequent Cox regression analyses (with all-cause mortality as competing risk), increasing ferritin concentrations were strongly associated with the development of new-onset HF ($P < 0.001$; *Table 3*). However, this association was lost in multivariable analyses. Interaction analyses showed a significant interaction between sex, ferritin levels, and new-onset HF (P for interaction = 0.032). When stratified by sex, this association persisted only in women ($P < 0.001$) and remained significant in all multivariable models. Adjusting for menstrual status did not alter the results. No interaction between menstrual status, ferritin levels, and development of new-onset HF was found in women ($P = 0.59$). Additional subgroup analyses in women demonstrated increasing ferritin levels to amplify risk for incident HF in almost every subgroup, but were significantly more predictive in women

Table 1 Baseline characteristics according to ferritin quartiles

Variables	Total	Q1	Q2	Q3	Q4	*P*-value for trend
n	6386	1636	1579	1576	1595	NA
Ferritin (μg/L), min–max	97 (1–1925)	27 (1–48)	71 (49–97)	130 (98–172)	257 (173–1925)	NA
Age (years)	53.1 ± 12.0	48.3 ± 10.9	53.3 ± 12.0	54.6 ± 11.7	56.4 ± 11.7	<0.001
Female sex (%)	50.7	82.0	56.6	40.5	23.1	<0.001
BMI (kg/m^2)	26.7 ± 4.3	25.5 ± 4.3	26.2 ± 4.3	26.9 ± 4.2	28.1 ± 4.1	<0.001
>30 kg/m^2 (%)	18.7	12.7	15.5	19.1	27.6	<0.001
Systolic BP (mmHg)	126.3 ± 18.8	119.5 ± 17.1	125.0 ± 18.7	128.5 ± 18.1	132 ± 18.6	<0.001
Heart rate (b.p.m.)	68.5 ± 10.0	68.9 ± 9.4	68.4 ± 10.3	68.4 ± 10.0	68.3 ± 10.3	0.022
LVH according to Cornell (%)	2.2	1.0	2.3	2.2	3.2	0.001
LBBB (%)	5.8	3.4	4.8	7.1	8.1	<0.001
Medical history (%)						
Smoking or quit <1 year	30.4	29.7	32.8	31.0	28.0	0.19
MI	2.9	2.0	2.5	3.1	4.2	<0.001
Stroke	0.9	0.9	1.1	0.6	0.9	0.68
Type 2 diabetes	7.8	5.0	6.3	7.8	12.1	<0.001
Hypercholesterolaemia	24.9	15.8	24.1	26.6	33.1	<0.001
Hypertension	31.2	19.1	27.8	34.4	43.7	<0.001
Anaemia	9.3	20.9	7.2	5.0	4.1	<0.001
Laboratory measurements						
Haemoglobin (g/dL)	13.7 ± 1.2	12.9 ± 1.2	13.7 ± 1.1	14.0 ± 1.1	14.3 ± 1.1	<0.001
MCV (fL)	90.4 ± 4.6	88.9 ± 5.3	90.7 ± 4.1	90.8 ± 4.2	91.3 ± 4.4	<0.001
Hepcidin (nmol/L)	3.1 (1.7–4.9)	1.1 (0.6–1.7)	2.6 (1.8–3.5)	3.8 (2.8–5.1)	5.6 (4.2–8.0)	<0.001
Serum iron (μmol/L)	15.9 ± 5.6	14.1 ± 6.3	16.0 ± 5.2	16.1 ± 5.1	17.2 ± 5.5	<0.001
TSAT (%)	24.8 ± 9.4	20.6 ± 9.5	25.0 ± 8.3	25.8 ± 8.4	28.2 ± 9.6	<0.001
Erythropoietin (IU/L)	7.8 (5.9–10.3)	8.6 (6.5–12.0)	7.7 (5.9–10.1)	7.5 (5.7–9.7)	7.3 (5.5–9.7)	<0.001
hs-CRP (mg/L)	1.4 (0.6–3.0)	1.0 (0.4–2.3)	1.3 (0.6–2.9)	1.4 (0.7–3.2)	1.8 (0.8–3.6)	<0.001
Cholesterol (mmol/L)	5.4 ± 1.0	5.1 ± 1.0	5.4 ± 1.0	5.5 ± 1.0	5.6 ± 1.1	<0.001
Glucose (mmol/L)	4.8 (4.4–5.3)	4.6 (4.3–5.1)	4.7 (4.4–5.2)	5.0 (4.5–5.4)	5.1 (4.6–5.6)	<0.001
Creatinine (μmol/L)	84.8 ± 19.1	78.9 ± 14.1	83.4 ± 16.9	86.5 ± 19.0	90.4 ± 23.6	<0.001
eGFR (mL/min/1.73 m^2)	89.6 ± 17.7	94.1 ± 17.1	89.9 ± 17.6	88.2 ± 17.4	86.2 ± 17.7	<0.001
<60 mL/min/1.73 m^2 (%)	5.7	3.2	5.5	6.7	7.7	<0.001
UAE (mg/24 h)	8.5 (6.0–15.1)	7.8 (5.7–12.4)	7.9 (5.8–13.9)	8.5 (6.1–16.0)	10.0 (6.7–20.3)	<0.001

Continuous variables are presented as mean ± standard deviation or as median (interquartile range) when non-normally distributed. Binary categorical variables are presented as percentages.

BMI, body mass index; BP, blood pressure; eGFR, estimated glomerular filtration rate; hs-CRP, high-sensitivity C-reactive protein; LBBB, left bundle branch block; LVH, left ventricular hypertrophy; MCV, mean corpuscular volume; MI, myocardial infarction; NA, not applicable; TSAT, transferrin saturation; UAE, urinary albumin excretion.

without anaemia, without microalbuminuria, and with an eGFR >60 mL/min/1.73 m^2 (Supplementary material online, *Figure S2*).

Since body iron stores are firmly regulated at the systemic level by hepcidin, we additionally explored the role of hepcidin and risk of new-onset HF. Similar observations regarding adjusted annual HF incidence were made for hepcidin, both overall and in women (*Figure 1*). Likewise, a strong association between levels of hepcidin and the development of new-onset HF was observed in women only (*P* for interaction = 0.045). Removing potential outliers (highest and lowest 1%) did not change the results. In subjects without prior MI and/or stroke, both ferritin and hepcidin levels remained strongly associated with the development of HF in women (Supplementary material online, *Table S4*). Sensitivity analyses in post-menopausal women showed ferritin—and not hepcidin—to remain predictive for new-onset HF (Supplementary material online, *Table S5*).

To assess whether the associations between ferritin levels and risk of new-onset HF were modified by other markers of iron homeostasis (TSAT and hepcidin), inflammation (hs-CRP), or the metabolic syndrome (BMI, glucose, or systolic blood pressure), the joint association of ferritin levels with either one of these six variables and the development of HF was examined. In these joint analyses, ferritin levels remained independently associated with new-onset HF in women in almost all stratified analyses (*Figure 2*).

Finally, 79 of the 199 subjects newly diagnosed with HF were women. Of these women, 37 developed HFrEF and 42 HFpEF. Unadjusted cause-specific hazard analyses demonstrated serum ferritin to be associated with either HFrEF or HFpEF, with no significant interaction between both entities (Supplementary material online, *Table S6*). Multivariable analyses showed that levels of ferritin remained strongly associated with an increased risk for HFpEF only. However, again no significant interaction was found.

Table 2 Multivariable regression beta-coefficients between cardiovasular risk factors and serum ferritin in men and women (selected after bootstrapping)

Variables	Men ($n = 3145$)			Women ($n = 3241$)		
	Standardized β	*T*	*P*-value	Standardized β	*T*	*P*-value
Menstruation (yes vs. no)	–	–	–	−0.129	−7.4	<0.001
BMI (per kg/m^2)	0.090	5.4	<0.001	–	–	–
Smoking (yes vs. no)	–	–	–	−0.035	−2.6	0.011
Haemoglobin (per g/dL)	0.108	6.5	<0.001	0.071	4.1	<0.001
MCV (per fL)	0.099	6.0	<0.001	0.124	7.6	<0.001
Log 2 hepcidin (nmol/L)	0.680	38.6	<0.001	0.625	30.4	<0.001
TSAT (per 5%)	0.094	5.1	<0.001	0.131	5.7	<0.001
Log 2 erythropoietin (IU/L)	–	–	–	−0.031	−2.0	0.042
Log 2 hs-CRP (mg/L)	–	–	–	0.059	3.5	<0.001
Cholesterol (mmol/L)	0.044	2.5	0.011	–	–	–
Log 2 glucose (mmol/L)	0.043	3.6	<0.001	0.052	4.4	<0.001
	R^2 total model		0.60	R^2 total model		0.74

BMI , body mass index; hs-CRP, high-sensitivity C-reactive protein; LVH, left ventricular hypertrophy; MCV, mean corpuscular volume; TSAT, transferrin saturation.

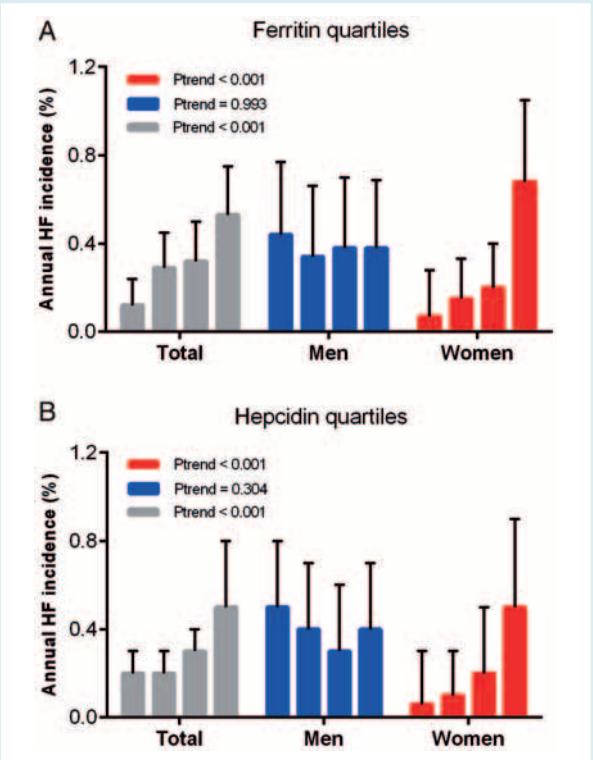

Figure 1 Total annual incidence of new-onset heart failure (HF) according to ferritin (*A*) and hepcidin (*B*) quartiles and stratified by sex.

Markers of iron homeostasis and risk for cardiovascular disease and all-cause mortality

A higher total annual incidence of new CV events and all-cause mortality was observed per increasing ferritin and hepcidin quartile (Supplementary material online, *Figure S3*). When stratified by sex, these observations only persisted in women. Multivariable fractional polynomials again showed the best fitting functional form between levels of ferritin or hepcidin and new CV events to be linear. Crude regression analyses showed increasing ferritin and hepcidin concentrations to be both strongly predictive for new CV events and all-cause mortality in the overall population and in women (both $P < 0.001$). However, age and multivariable analyses were non-significant. No interaction between variables was demonstrated.

Discussion

In this contemporary, prospective, population-based cohort, increasing ferritin levels amplify the risk for new-onset HF in women, but not in men. This relationship is independent of established conventional CV and HF risk factors and the development of CV events over time. These observations provide evidence that increased ferritin levels are an independent risk factor for the development of HF in women and might play a direct or indirect role in the pathogenic mechanism leading to HF.

How do elevated iron stores relate to incident heart failure?

Whereas iron deficiency has detrimental effects on outcome in established HF,[21–23] the role of iron markers or markers of erythropoiesis in the development of HF has only been investigated to some extent.[24,25] Previous literature suggested a U-shaped relationship between iron stores and new-onset HF.[3,4] In the present study, we observed a linear relationship between increasing ferritin levels and the development of HF. Excess body iron is known to accumulate in organs such as the heart and lead to cardiomyopathy. The generation of reactive oxygen species (ROS) in mitochondria is believed to be the most important pathogenic

Table 3 Levels of ferritin and hepcidin and risk for incident heart failure with all-cause mortality as a competing risk

Ferritin (per doubling)	sHR	95% CI	Harrell's C	*P*-value
Total population ($n=6386$)[a]				
Univariable	1.48	1.30–1.69	0.64	<0.001
Model 1	1.24	1.05–1.46	0.83	0.011
Model 2	1.06	0.89–1.26	0.88	0.45
Men ($n=3145$)				
Univariable	1.00	0.81–1.23	0.52	0.98
Model 1	1.06	0.87–1.29	0.82	0.54
Model 2	0.92	0.75–1.14	0.87	0.45
Women ($n=3241$)				
Univariable	2.07	1.63–2.61	0.74	<0.001
Model 1	1.57	1.20–2.05	0.83	0.001
Model 1A	1.69	1.26–2.26	0.79	<0.001
Model 2	1.35	1.02–1.79	0.87	0.038
Model 3	1.39	1.05–1.86	0.87	0.024
Hepcidin (per doubling)	**HR**	**95% CI**	**Harrell's C**	***P*-value**
Total population ($n=6386$)[b]				
Univariable	1.45	1.23–1.72	0.61	<0.001
Model 1	1.20	0.98–1.45	0.83	0.07
Model 2	1.09	0.92–1.30	0.88	0.39
Men ($n=3145$)				
Univariable	0.96	0.79–1.16	0.54	0.76
Model 1	1.00	0.81–1.25	0.82	0.94
Model 2	0.95	0.74–1.21	0.87	0.49
Women ($n=3241$)				
Univariable	2.10	1.63–2.70	0.75	<0.001
Model 1	1.59	1.17–2.17	0.84	0.003
Model 1A	1.68	1.19–2.37	0.79	0.003
Model 2	1.44	1.03–2.05	0.87	0.039
Model 3	1.60	1.09–2.37	0.88	0.018

CI , confidence interval; HR, hazard ratio; sHR, subhazard ratio.
[a]*P* for interaction ferritin, sex, and new-onset heart failure = 0.032.
[b]*P* for interaction hepcidin, sex, and new-onset heart failure = 0.045.
Model 1 is adjusted for age (per 10 years) and sex (in total population).
Model 1A is adjusted for menstrual state instead of age.
Model 2 is adjusted for model 1 + cardiovascular and heart failure risk factors: body mass index, current smoking, the presence of diabetes, hypertension, hypercholesterolaemia, history of myocardial infarction or stroke, left ventricular hypertrophy, renal function, and levels of haemoglobin, erythropoietin, high-sensitivity C-reactive protein, and urinary albumin excretion.
Model 3 is adjusted for model 2 + time-varying incident cardiovascular events.

pathway determining cardiomyocyte damage. Production of ROS causes lipid peroxidation and DNA damage, leading to cell death, fibrosis, and eventually cardiac dysfunction. However, iron overload is only observed when the binding capacity of transferrin is fully saturated and non-transferrin bound iron is formed. Interestingly, joint analyses showed an association between serum ferritin and new-onset HF in all TSAT tertiles, suggesting that another pathway linking increased ferritin levels with cardiomyocyte damage may be involved.

Iron-mediated cell damage does not only occur under conditions of iron overload. It has been proposed that iron maldistribution among organs, tissues, and cellular compartments can also attenuate cell integrity and cell life. Dysmetabolic hyperferritinaemia, meaning elevated ferritin levels with normal TSAT, is commonly observed in clinical practice and has been associated with insulin resistance, obesity, hypertension, and other manifestations of the metabolic syndrome.[26] During circumstances of subclinical systemic inflammation, iron efflux in organs may be halted due to increased hepcidin production and subsequent internalization and degradation of its receptor, the iron exporter ferroportin.[27] Therefore, elevated ferritin levels (and hepcidin) might be a reflection of a low-grade inflammatory response to another pathophysiological process (e.g. metabolic syndrome), causally responsible for the development of HF.[28] Supporting this hypothesis is the fact that we observed a positive trend in blood pressure, BMI, and levels of glucose, cholesterol, and hs-CRP per increasing ferritin quartile. Additionally, strong associations between ferritin and levels of hepcidin, hs-CRP, and glucose in women were observed. To minimize for potential confounding, we adjusted for the presence of obesity, diabetes, hypertension, hypercholesterolaemia, and inflammation in our multivariable analyses. Moreover, joint analyses showed increasing ferritin levels to remain predictive for incident HF in almost all tertiles. Nevertheless, to what extent elevated ferritin levels truly reflect iron stores (inflammation and oxidative stress may complicate the picture) is unclear. Moreover, the exact mechanism linking increased ferritin levels, the iron-regulatory hormone hepcidin, and the development of HF is still not completely understood.

To our surprise, the association between increasing ferritin levels and new-onset HF was only present in women. It is known that some traditional CV risk factors may have a different impact on males compared with females.[29] Additional literature also suggests that the link between ferritin, hepcidin, and dysmetabolic features may be particularly relevant in women.[30,31] The role of sex hormones in the regulation of ferritin and hepcidin expression may also be responsible to a certain extent for the sex-related differences observed in the present study and has recently been studied in animal models.[32] Yet, although we adjusted for menopausal status and other established CV/metabolic risk factors, levels of ferritin remained associated with incident HF in women.

Finally, a stronger association between levels of ferritin and the development of HFpEF was observed in women. To date there is a limited understanding of the underlying pathophysiology of HFpEF. A novel concept in the pathogenesis of HFpEF was proposed involving the role of co-morbidities (e.g. hypertension or diabetes) to induce a subclinical inflammatory state, eventually leading to microvascular endothelial inflammation and subsequent stiffening and diastolic dysfunction.[33] Given the aforementioned relationship between dysmetabolic hyperferritinaemia and diabetes or metabolic syndrome, iron misdistribution and consecutive oxidative stress might play a direct or indirect role in the development of HFpEF. However, no significant interaction between both HF entities was observed, and the number of HFpEF cases in the present study is low. Therefore, these results need cautious interpretation and merit further investigation.

Markers of iron status and risk of cardiovascular events

In the present study, we did not observe an association between ferritin or hepcidin concentrations and development of CV

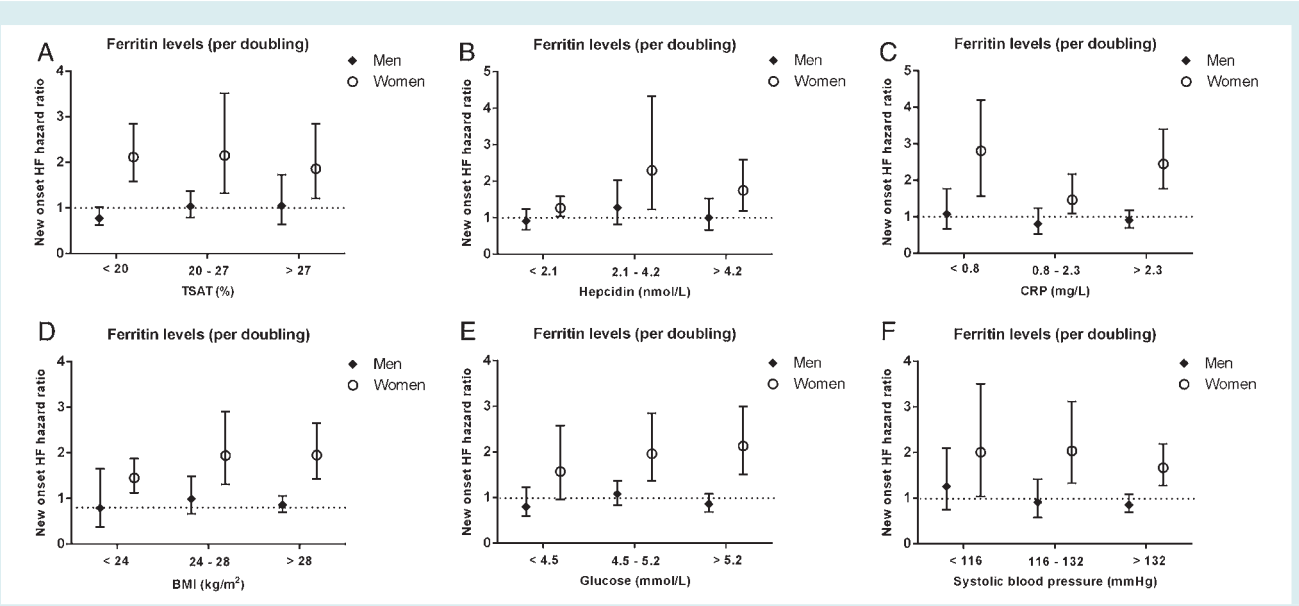

Figure 2 Sex-stratified risk for new-onset heart failure according to the joint classification of ferritin levels and tertiles of transferrin saturation (TSAT; *A*), hepcidin (*B*), high-sensitivity C-reactive protein (CRP; *C*), body mass index (BMI; *D*), glucose (*E*), or systolic blood pressure (*F*).

events or all-cause mortality in multivariable analyses. It has been hypothesized that both elevated and decreased markers of iron status enhance the risk for CV disease.[34,35] However, conflicting results have also been reported.[36] Furthermore, studies in haemodialysis patients or women from the general population have suggested a role for elevated hepcidin levels in the pathogenesis of atherosclerosis.[37–39] In contrast, Kautz and colleagues did not detect any increase in hepatic hepcidin expression during progression of atherosclerosis or atherosclerotic plaque size in mice with elevated macrophage iron.[6] A recently published population-based study also found no relationship between hepcidin or the hepcidin-to-ferritin ratio and prevalent carotid atherosclerosis or incident CV events.[7] Future population-based studies are needed to establish finally whether an abnormal iron homeostasis is associated with the development of CV events in the general population.

Implications for clinical practice

Although the present associations do not prove causality between iron stores and incident HF, the present study provides evidence that increased ferritin levels may directly or indirectly be associated with the pathogenesis of new-onset HF in women. Identification of subjects at risk for HF using biomarkers alongside clinical characteristics has gained interest over the years. Markers of iron homeostasis may provide additional information to the clinician regarding aetiology, clinical risk (in our case HF), and disease severity. Secondly, ferritin is a widely available routine marker and a relatively inexpensive measurement. Finally, elimination of iron may confer a beneficial effect. Iron removal, by means of phlebotomy, improved coronary vascular function in patients with type 2 diabetes and endothelial function in patients with known CAD.[40,41] Still, the findings of this study need to be validated in other population-based studies.

Strengths and limitations

The large size of this contemporary, prospective community-based cohort, long follow-up period, and thorough validation of outcome parameters are strengths of this study. Furthermore, all blood samples were taken in the morning, minimizing the influence of circadian rhythm on markers of iron status. The present study is limited by the fact that the subjects from the PREVEND study are predominantly Caucasian and our results cannot therefore be extrapolated to subjects from other ethnicities. Secondly, our analyses rely on one baseline measurement of iron markers and hepcidin, which is used as a proxy for the concentration in the years before and after this measurement. Therefore, we cannot comment on the effects of changes in markers over time. More population-based studies with serial measurements over time are warranted. Thirdly, long-term storage at −80 °C might be associated, to some extent, with less precise measurements of hepcidin. A recent paper by Laarakkers and co-workers investigated the stability of hepcidin during long-term storage.[42] The authors concluded that hepcidin results are not changed during 2 years of storage at −80 °C. However, after 2 years of storage at −80 °C, hepcidin results may become less precise. Although we believe that average results for a population may not be affected, perhaps existing differences in hepcidin levels between groups, or correlations of hepcidin with other parameters might be more difficult to assess. Finally, the PREVEND cohort is enriched for increased UAE. For this reason, we corrected for study design by conducting a design-based analysis. Furthermore,

compared with the Framingham cohort, UAE was not higher in PREVEND.[43]

Conclusions

In conclusion, increased ferritin levels independently enhance the risk for new-onset HF in apparently healthy women. These findings provide evidence that elevated ferritin concentrations may be directly or indirectly involved in the pathogenesis of new-onset HF in women in the community.

Supplementary Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Baseline characteristics according to ferritin quartiles in men and women.

Table S2. Univariable regression β-coefficients between cardiovascular risk factors and ferritin in men and women.

Table S3. Multivariable regression β-coefficients between cardiovascular risk factors and serum ferritin in men and women without prior myocardial infarction and/or stroke (selected after bootstrapping).

Table S4. Ferritin and hepcidin levels and incident heart failure with death as competing risk in subjects without prior myocardial infarction and/or stroke.

Table S5. Hepcidin and ferritin levels and incident heart failure with death as competing risk in post-menopausal women.

Table S6. Levels of ferritin and cause-specific risk for heart failure with a reduced or preserved ejection fraction in women.

Figure S1. Receiver operating characteristic curves and areas under the curve of serum iron, transferrin saturation, and ferritin in predicting new-onset heart failure, cardiovascular events, cardiac events, and all-cause mortality.

Figure S2. Ferritin levels and risk for new-onset heart failure among subgroups in women.

Figure S3. Total annual incidence of cardiovascular events and all-cause mortality according to ferritin and hepcidin quartiles and stratified by sex.

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