

Iron homeostasis in heart transplant recipients randomized to ferric derisomaltose or placebo

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TWEET: Definition of iron deficiency in heart failure is not suitable for heart transplant recipients.

Abstract

Introduction: The randomized IronIC trial evaluated the effect of intravenous ferric derisomaltose on physical capacity in iron-deficient, maintenance heart transplant (HTx) recipients. Iron deficiency was defined as in heart failure with high cut-points for ferritin to compensate for inflammation. However, intravenous iron did not improve physical capacity except in patients with ferritin $<30 \mu\text{g/L}$. We aimed to explore determinants of iron status in the 102 IronIC participants to better define iron deficiency in the HTx population.

Methods: We assessed key governors of iron homeostasis, such as hepcidin, soluble transferrin receptor (sTfR), and interleukin-6 (IL-6). We also measured growth factors and inflammatory markers with relevance for iron metabolism. The results were compared to those of 21 healthy controls.

Results: Hepcidin did not differ between HTx recipients and controls, even though markers of inflammation were modestly elevated. However, HTx recipients with ferritin $<30 \mu\text{g/L}$ or sTfR above the reference range had significantly reduced hepcidin levels suggestive of true iron deficiency. In these patients, intravenous iron improved peak oxygen uptake. Hepcidin correlated positively with ferritin and negatively with sTfR.

Conclusion: HTx recipients with iron deficiency as defined in heart failure do not have elevated hepcidin levels, although inflammatory markers are modestly increased. The high ferritin cut-offs used in heart failure may not be suitable to define iron deficiency in the HTx population. We suggest that hepcidin and sTfR should be measured to identify patients with true iron deficiency, who might benefit from treatment with intravenous iron.

KEYWORDS

heart transplant, inflammation, intravenous iron, iron metabolism

1 | INTRODUCTION

Heart transplant (HTx) recipients have reduced exercise capacity compared to gender- and age-matched healthy individuals.^{1,2} The impaired physical performance observed in these patients is probably multifactorial and may partly involve iron deficiency, usually reflected by low circulating levels of ferritin. However, iron deficiency is difficult to identify in chronic illness because ferritin levels are also influenced by inflammation.³

Hepcidin is the main governor of iron homeostasis. The expression of hepcidin is regulated by iron status.⁴ Iron deficiency leads to less hepcidin and subsequently increased absorption of dietary iron, and increased efflux of intracellular iron. This reduces iron stores, reflected by lower circulating levels of ferritin, while available iron in plasma increases. Vice versa, an abundance of iron induces the expression of hepcidin, intracellular iron stores are replenished, and circulating ferritin increases. However, hepcidin is also upregulated by the activation of inflammatory pathways, and in particular interleukin (IL)-6, which precipitates the anemia associated with chronic diseases. On the other hand, growth derived factor-15 (GDF-15) and hypoxia-inducible factor-1 α (HIF-1 α) attenuate hepcidin synthesis.^{5,6} In addition, it has been suggested that hepcidin is suppressed by heme-oxygenase-1 (HO-1), an enzyme crucial for iron recycling in senescent erythrocytes.⁷

Patients with chronic inflammation may have iron deficiency due to IL-6-induced hepcidin expression, while having normal or elevated ferritin. These patients might benefit from iron supplements at higher ferritin cut-off values than those applied in the general population. Alternatively, transferrin saturation of <20% may be used to define iron deficiency in such patients.⁸ Soluble transferrin receptor (sTfR) reflects tissue iron supply and is not substantially affected by inflammation,⁹ and may therefore be a better marker of iron status when inflammation is present.¹⁰ The relative amount of sTfR to ferritin, as reflected by the sTfR/log[ferritin] (sTfR/F) index, may be an even better indicator of iron depletion than sTfR alone.^{11,12} Moreover, hepcidin itself may be a key parameter for distinguishing between true iron deficiency and iron deficiency that is secondary to chronic inflammation, with low and high hepcidin levels, respectively.¹³

Intravenous iron supplement improves exercise capacity in patients with heart failure and iron deficiency as defined by ferritin <100 μ g/L or ferritin 100–300 μ g/L in combination with transferrin saturation of <20%.^{14,15} These cut-points are therefore used to define iron deficiency in heart failure.¹⁶ We have previously shown that by this definition, iron deficiency is highly prevalent in maintenance HTx recipients.¹⁷ However, in the Intravenous Iron supplement for Iron deficiency in Cardiac transplant recipients (IronIC) trial (clinicaltrials.gov NCT03662789), intravenous iron did not improve physical capacity in HTx recipients with this broad definition of iron deficiency.¹⁸

In this sub-study of the IronIC trial, we aimed to assess iron metabolism in HTx recipients with iron deficiency as defined in heart failure and compare results to those of healthy, non-transplanted

controls. Based on the neutral effect of intravenous iron in the HTx recipients, we hypothesized that iron metabolism would not be significantly disturbed in these patients. We also sought to explore the clinical usefulness of different definitions of iron deficiency in HTx recipients with particular focus on hepcidin and sTfR levels, and their association with markers of inflammation.

2 | METHODS

2.1 | The IronIC trial

The randomized, controlled, double-blinded IronIC trial was conducted at Oslo University Hospital, Rikshospitalet and has been reported in detail.¹⁸ In summary, 102 HTx recipients with ferritin <100 μ g/L or ferritin 100–300 μ g/L in combination with transferrin saturation of <20% were randomized in a 1:1 manner to treatment with ferric derisomaltose 20 mg/kg, or placebo. The primary endpoint was peak oxygen consumption. We regarded a between-group difference of 1.5 ml/kg/min as clinically significant. Patients were assessed at baseline, before randomization, and at 6 months' follow-up. At 6 months, there was no significant between-group difference in peak oxygen consumption, and we concluded that intravenous iron supplement did not improve exercise capacity in HTx recipients with iron deficiency as defined in heart failure. However, peak oxygen consumption did improve in a small subgroup of patients with serum ferritin <30 μ g/L. The trial was approved by the Norwegian South-East Regional Ethics Committee and conducted in compliance with the Declaration of Helsinki and rules for Good Clinical Practice.

2.2 | Patients and definitions

Patients in the IronIC trial had iron deficiency as defined in heart failure, were between 18 and 80 years old, and were transplanted at least 1 year before enrollment in the trial. The complete inclusion and exclusion criteria have been published elsewhere.¹⁸ For this substudy, we dichotomized the patient cohort by cut-points reflecting different definitions of iron deficiency: ferritin <100 μ g/L; ferritin <30 μ g/L; transferrin saturation of <20%; sTfR above the reference range; C-reactive protein (CRP) >5 mg/L; and the index of sTfR divided by the logarithmic value of ferritin >2.1 (sTfR/F) >2.1. The 2.1 cut-point reflects the median sTfR/F index in patients who improved their peak oxygen consumption after randomization to intravenous iron in the IronIC trial.

2.3 | Controls

For comparison, we used 21 healthy, non-transplanted controls enrolled between November 2009 and November 2010. The control subjects had no history of chronic disease and did not use any medications.

2.4 | Biobank samples

Blood samples were drawn at baseline, before randomization, and at 6 months follow-up. We collected blood in six tubes containing ethylenediaminetetraacetic acid (EDTA), and three tubes without additives. The EDTA tubes were immediately put on ice and centrifuged at 3700 revolutions per minute for 20 min, within 20 min after collection. Tubes without additives were left in room temperature for coagulation in 1–2 h prior to centrifugation at 3500 revolutions per minute for 15 min. The resulting supernatants were stored at -80°C . Blood from the controls was collected and treated in the same manner as the trial participants.

2.5 | Biochemical analysis

In the IronIC trial, we measured creatinine, CRP, N-terminal pro-B-type natriuretic peptide (NT-proBNP), hemoglobin, serum ferritin, and transferrin saturation. We also analyzed serum-iron and sTfR using cobas c701/701 (Roche Diagnostic, Mannheim, Germany), and the Tina quant sTfR assay (Roche Diagnostic), respectively. Anemia was defined according to the WHO recommendations; hemoglobin <130 g/L in men, and hemoglobin <120 g/L in women.¹⁹

The biobank samples were used to evaluate biomarkers related to iron homeostasis. The results were compared to those of the controls. We measured hepcidin, IL-6, pentraxin related protein 3 (PTX3), GDF-15, HIF-1 α , and HO-1. Enzyme-linked immunosorbent assays were used to measure levels of hepcidin (catalogue #DY8307-05; R&D Systems, Minneapolis, MN); IL-6 (catalogue #K151QXD-2; Meso Scale Diagnostics; Gaithersburg, MD); PTX3 (catalogue # DY1826; R&D Systems); GDF-15 (catalogue #DY957; R&D Systems), HIF-1 α (catalogue # EHIF1A5; Thermo Fischer Scientific, Agawam, MA) and HO-1 (catalogue #MBS2099158; MyBioSource Inc, San Diego, CA). To compare levels of sTfR between patients and controls, we remeasured sTfR with an enzyme-linked immunosorbent assay (catalogue #DY2474; R&D Systems).

2.6 | Statistics

We performed all statistical analyses using SPSS version 27 (IBM Corp.). Normally distributed data are presented as mean \pm standard deviation and skewed data is presented as median (interquartile range). Categorical variables are presented as frequency counts and percentages. We used the independent *t*-test, Chi-Square and Fischer's exact test for comparison between groups. Skewed variables were ln-transformed to meet test assumptions. The Mann-Whitney *U*-test was performed if ln-transformation failed to produce an approximately normal distribution. Multiple analysis of covariance (MANCOVA) was used for comparison between groups when corrections for age and creatinine were appropriate. Pearson's correlation was performed to evaluate the relationship between hepcidin and ferritin and selected

variables. The data that supports the findings of this study is available on request from the corresponding author, Kaspar Broch. The data is not publicly available because they contains information that could compromise the privacy of research participants.

3 | RESULTS

One hundred and two patients were enrolled in the IronIC trial. Biobank samples were available for 97 patients at baseline and 94 patients at follow-up. Demographics for patients and controls are presented in Table 1. The median time since HTx was 8 (4–13) years, and the peak oxygen consumption at baseline was 23 ± 7 ml/kg/min. Participants in the IronIC trial were slightly younger and had higher body mass index, CRP, creatinine, and NT-pro-BNP than controls. Anemia was more prevalent than in the control group. The subgroup demographics (Table 2) were similar to those of the total patient population.

3.1 | Hepcidin

Hepcidin did not differ significantly between patients and controls. However, hepcidin levels in the HTx recipients correlated positively with ferritin and negatively with sTfR (Table 3). Patients with ferritin <30 $\mu\text{g/L}$ and patients with sTfR $>$ the reference value had significantly lower levels of hepcidin than the control group (Table 2). Patients with CRP >5 mg/L had the highest hepcidin levels, but their hepcidin levels did not differ significantly from those of the controls. Hepcidin increased significantly from baseline to follow-up in patients who were randomized to receive intravenous iron (40 [15–80] to 93 [62–163] ng/ml; $p < .001$); whereas there was no change from baseline to follow-up in patients randomized to placebo (19 [9–58] ng/mL at baseline vs. 15 [6–42] ng/ml at follow-up; $p = .18$; Figure 1).

3.2 | Soluble transferrin receptor

Levels of sTfR were not elevated in the HTx group as a whole but were higher than in control subjects in patients with transferrin saturation $<20\%$ and in patients with ferritin <30 $\mu\text{g/L}$. Levels of sTfR fell significantly from baseline to follow-up in patients who were randomized to intravenous iron ($1.5 \pm .5$ to $1.3 \pm .3$, $p < .001$).

3.3 | Inflammatory markers

Interleukin-6 (IL-6) and CRP were significantly higher in the patients than in controls (Table 1). CRP was particularly high in patients with low transferrin saturation and in patients with elevated levels of sTfR (Table 2). Pentraxin related protein 3 was increased in all subgroups of patients. There were no correlations between hepcidin and PTX3, CRP or IL-6 in the HTx recipients.

TABLE 1 Demographics and biochemistry in patients and controls

Demographics	Controls (n = 21)	HTx recipients (n = 97)	p for difference
Clinical characteristics			
Age, years	62 ± 6	55 ± 14	<.001 ^a
Male gender – no (%)	12 (57)	64 (66)	.46 ^b
Body mass index – kg/m ²	24.9 ± 2.8	26.8 ± 4.6	.07 ^a
Routine biochemistry			
Hemoglobin – g/L	143 ± 10	137 ± 15	.10 ^a
Anemia – no (%)	0 (0)	23 (24)	.01 ^c
C-Reactive Protein – mg/L	.9 (.6–1.8)	1.7 (.8–5.1)	.04 ^d
Creatinine – μmol/L	74 ± 11	104 ± 29	<.001 ^a
N-terminal-pro-B-type natriuretic peptide – ng/L	66 (32–114)	354 (184–783)	<.001 ^a
Conventional parameters reflecting iron status			
Ferritin – μg/L	NA	64 (42–89)	NA
Transferrin saturation – %	NA	22 ± 9	NA
s-Iron – μmol/L	NA	12.0 (9.0–17.0)	NA
Biobank analyses			
Hepcidin – ng/ml	26 (11–46)	31 (10–71)	.50 ^e
Soluble Transferrin Receptor – ng/ml	1.03 (.95–1.36)	1.45 (1.15–1.72)	.07 ^e
Interleukin-6 – pg/ml	.67 (.47–1.09)	1.30 (.75–3.01)	.03 ^e
Pentraxin related protein 3 – ng/ml	3.47 (2.79–4.33)	6.11 (4.10–9.21)	<.001 ^e
Growth Derived Factor-15 – pg/ml	425 (371–561)	850 (471–1021)	.04 ^e
Hypoxia-Inducible Factor-1α – pg/ml	155 (70–327)	137 (69–414)	.75 ^e
Heme Oxygenase-1 – ng/ml	3.99 (2.85–4.76)	4.11 (3.30–6.09)	.47 ^e

Note: Demographics and biochemistry in controls and at baseline in the heart transplant recipients in the IronIC trial, who had serum ferritin <100 μg/L or ferritin 100–300 μg/L in combination with transferrin saturation of <20%. Numbers are given as means ± standard deviation, median (interquartile range) or no (%).

Abbreviations: HTx, heart transplant; NA, Non-applicable.

^at-test.

^bChi Square.

^cFischer's exact test.

^dMann-Whitney U.

^eMANCOVA corrected for age and creatinine.

3.4 | Growth derived factor-15, hypoxia-inducible factor-1α, and heme-oxygenase-1

GDF-15 was higher in the patients than in controls ($p = .04$). However, there were no correlations between GDF-15 and hepcidin or ferritin. GDF-15 increased significantly from 578 (455–969) pg/ml at baseline to 631 (459–1119) pg/ml at follow up in patients who were randomized to iron ($p = .02$). HIF-1α and HO-1 levels did not differ between the HTx recipients and controls. Both of these biomarkers were correlated with ferritin, but not with hepcidin (Table 3).

3.5 | Immunosuppressant therapy

Seventy-one patients received calcineurin inhibitors (CNIs) and 33 patients received mammalian target of rapamycin (mTOR) inhibitors,

seven of whom received both CNIs and mTOR inhibitors. Patients treated with mTOR inhibitors had higher CRP ($p < .001$), IL-6 ($p = .03$), and sTfR ($p = .02$) and lower transferrin saturation ($p = .04$) compared to patients receiving CNIs. There was no between-group difference in ferritin and hepcidin.

3.6 | Peak oxygen uptake

Overall, peak oxygen consumption did not improve with intravenous iron in the IronIC trial. However, in HTx recipients with ferritin <30 μg/L, intravenous iron improved peak oxygen uptake by 4.4 ml/kg/min (95% confidence interval .01–8.7, $p = .0495$). Retrospective analyses revealed that intravenous iron was associated with increased oxygen uptake in the 16 patients with sTfR above the reference range, in whom peak oxygen consumption increased

TABLE 2 Demographics and biochemistry in controls and subgroups of patients

	Controls n = 21	F < 100 n = 86	T _{sat} < 20 n = 50	F < 30 n = 13	sTfR > ref n = 16	sTfR/F > 2.1 n = 36	CRP > 5 n = 26
Clinical characteristics							
Age - years	62 ± 6	54 ± 14*	57 ± 12*	54 ± 13*	59 ± 11	57 ± 13	59 ± 13
Male gender - no (%)	12 (57)	54 (63)	36 (72)	7 (54)	10 (63)	22 (61)	19 (73)
BMI - kg/m ²	24.9 ± 2.8	27.1 ± 5.1*	27.6 ± 4.5*	27.2 ± 4.2	29.5 ± 4.4*	28.3 ± 5.2*	28.7 ± 5.3*
Routine biochemistry							
Hemoglobin - g/L	143 ± 10	137 ± 15	134 ± 14*	134 ± 1.3*	132 ± 16*	135 ± 15*	132 ± 13*
Anemia - no (%)	0 (0)	20 (23)*	15 (30)*	1 (8)	4 (25)*	9 (25)*	11 (42)*
C-reactive protein - mg/L	.9 (6-1.8)	1.6 (8-4.6)	2.7* (1.5-7.5)	1.5 (1.9-3.9)	3.6* (1.8-6.6)	2.6* (1.1-5.4)	8.1* (5.7-17.5)
Creatinine - μmol/L	74 ± 11	104 ± 29*	106 ± 29*	94 ± 22*	122 ± 42*	113 ± 37*	114 ± 37*
NT-proBNP - ng/L	66 (32-115)	360* (191-796)	373* (185-809)	392* (227-560)	399.0* (197-1405)	433* (274-801)	400* (202-1207)
Conventional parameters reflecting iron status							
Ferritin - μg/L	NA	60 (40-79)	60 (32-99)	27 (24-29)	41 (27-71)	45 (29-67)	75 (60-96)
Transferrin saturation - %	NA	22 ± 9	15 ± 3	15 ± 7	14 ± 5	16 ± 6	18 ± 7
s-Iron - μmol/l	NA	130 (10.0-17.0)	9.0 (8.0-11.0)	9.0 (6.5-12.0)	8.5 (6.3-11.8)	9.0 (8.0-11.8)	9.0 (7.8-14.0)
Biobank analyses							
Hepcidin - ng/ml	26.0 (10.9-46.2)	23.9 (9.4-68.1)	25.0 (7.6-59.0)	3.4* (1.3-12.9)	3.8* (2.1-35.0)	18.8 (3.0-58.4)	42.6 (10.0-77.7)
sTfR - ng/ml	1.03 (0.95-1.36)	1.46 (1.15-1.75)	1.50* (1.29-1.77)	1.62* (1.35-2.55)	2.40* (2.02-2.88)	1.74* (1.51-2.27)	1.42 (1.15-1.76)
IL-6 - pg/ml	.67 (47-1.09)	1.21 (0.75-2.80)	1.44* (.88-3.80)	.89 (.75-1.52)	2.41 (1.18-4.53)	1.59 (.76-3.07)	2.91* (1.18-4.17)
PTX3 - ng/ml	3.47 (2.79-4.33)	6.53* (4.27-9.12)	6.47* (4.15-9.51)	7.45* (4.34-10.17)	8.03* (6.78-10.83)	8.03* (5.04-10.01)	6.24* (3.69-7.46)
GDF-15 - pg/ml	425 (371-561)	664* (468-1043)	728 (510-964)	582 (402-824)	914 (635-1838)	732 (485-1117)	823 (552-1457)
HIF-1α - pg/ml	155 (70-327)	138 (69-437)	118 (69-339)	111 (79-440)	95 (67-1024)	105 (67-375)	79 (61-219)
HO-1 - ng/ml	3.9 (2.85-4.76)	3.97 (3.20-5.80)	4.38 (3.56-6.69)	3.94 (3.09-6.78)	5.06 (3.94-7.09)	4.28 (3.30-6.62)	6.55* (4.02-8.18)

Note: Demographics and biochemistry in controls in and subgroups of patients with ferritin <100 μg/L (F < 100); transferrin saturation <20% (T_{sat} < 20); ferritin <30 μg/L (F < 30); soluble transferrin receptor > reference range (sTfR > ref); soluble transferrin receptor/log ferritin > 2.1 (sTfR/F > 2.1); or C-reactive protein > 5 mg/L (CRP > 5). Values are presented as: mean ± standard deviation; median (interquartile range); and numbers (%).

Abbreviations: GDF-15, growth derived factor-15; HIF-1α, hypoxia inducible factor-1α; HO-1, heme oxygenase-1; IL-6, interleukin-6; NA, not applicable; NT-pro-BNP, N-terminal-pro-B-type natriuretic peptide; PTX3, pentraxin 3; sTfR, soluble transferrin receptor.

*Differs from control group at a p-value < .05. Values are corrected for age and/or creatinine when appropriate, in comparison with controls.

TABLE 3 Correlations between potential determinants of iron metabolism and hepcidin and ferritin

	Hepcidin		Ferritin	
	Pearsons r	p	Pearsons r	p
Hepcidin			.39	<.001*
Ferritin	.39	<.001*		
Soluble transferrin receptor	-.31	.002*	-.19	.06
Interleukin-6	.04	.67	.20	.05
C-reactive protein	.06	.54	.20	.05
Pentraxin 3	-.01	.92	.11	.30
Growth Derived Factor -15	-.06	.55	.10	.32
Hypoxia-Inducible Factor-1 α	.11	.28	-.21	.04*
Heme Oxygenase-1	.10	.34	.28	.005*

Note: Correlations between potential determinants of iron metabolism and hepcidin and ferritin in heart transplant recipients with ferritin <100 $\mu\text{g/L}$ or ferritin 100–300 $\mu\text{g/L}$ in combination with transferrin saturation of <20%. *p*-Values < .05 have been highlighted with an asterisk.

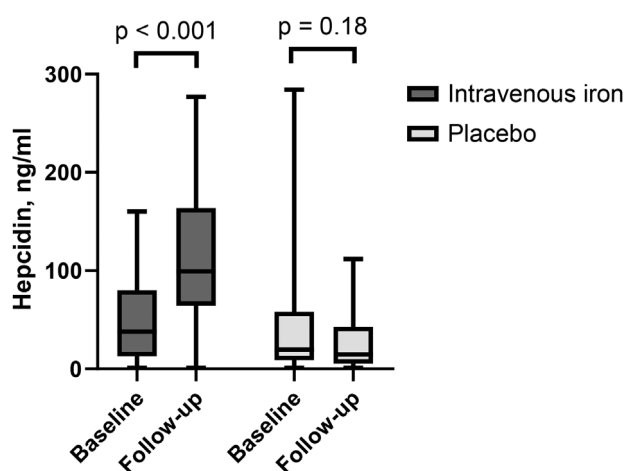


FIGURE 1 Hepcidin at baseline and at follow-up in heart transplant (HTx) recipients treated with intravenous iron or placebo. Hepcidin at baseline and at follow-up 6 months after randomization to intravenous iron derisomaltose in HTx recipients with ferritin <100 $\mu\text{g/L}$ or ferritin 100–300 $\mu\text{g/L}$ in combination with transferrin saturation of <20%. Boxes are 25–75 percentiles; whiskers are minimum values to maximum values. Hepcidin increased significantly in patients randomized to intravenous iron derisomaltose but did not change in patients randomized to matching placebo.

by 2.6 ml/kg/min (95% confidence interval .2–4.9, $p = .04$) (Central illustration). Moreover, in the 36 patients with sTfR/F > 2.1, peak oxygen uptake increased by 2.2 ml/kg/min (95% confidence interval .04–4.3, $p = .046$). Levels of hepcidin, CRP, GDF-15 or HO-1 were not related to the response in peak oxygen uptake to intravenous iron.

4 | DISCUSSION

We have recently shown that intravenous iron supplement did not improve physical capacity in HTx recipients with iron deficiency as defined in chronic heart failure.¹⁸ In this study, we show that there was a beneficial effect of intravenous iron supplementation on peak oxygen consumption in HTx recipients with sTfR above the normal reference range, as well as in patients with ferritin levels <30 $\mu\text{g/L}$. These patients had significantly reduced hepcidin levels, suggestive of true iron deficiency. Our findings suggest that carefully selected subgroups of HTx recipients could benefit from intravenous iron supplementation based on measurements of ferritin, sTfR and hepcidin.

Inflammation induces hepcidin expression with subsequent functional iron deficiency, putatively as a protective mechanism against iron-dependent microorganisms.²⁰ Although HTx recipients with CRP > 5 mg/L had the highest hepcidin levels, hepcidin did not correlate with markers of inflammation, suggesting that the inflammation was not sufficient to significantly impact iron metabolism in these patients. In other words, although HTx recipients have low-grade systemic inflammation, this inflammation is not sufficient to promote a hepcidin response. On the contrary, the significant increase in hepcidin observed in the patients who received intravenous iron suggests that the main regulator of hepcidin in HTx recipients is iron status. While the relatively high average peak oxygen consumption at baseline may explain why iron supplement did not augment exercise capacity in the IronIC trial, an alternative explanation may be that most of the patient were not truly iron deficient. Indeed, patients with clear signs of iron deficiency, as reflected by very low ferritin, high sTfR and low levels of hepcidin, improved their peak oxygen consumption after treatment with intravenous iron.

In heart failure, patients in New York Heart Association functional class IV have the highest levels of IL-6.^{21,22} However, the association between IL-6 and hepcidin in heart failure is unclear.^{21,23} On the other hand, intravenous iron supplement has been shown to improve clinical performance in patients with heart failure and iron deficiency defined by higher ferritin cut-offs.^{14,15,24} This suggests that ferritin is spuriously elevated in heart failure, and that patients with heart failure lack iron even though circulating levels of ferritin are not grossly reduced. The conflicting associations between IL-6 and hepcidin might be a result of confounding factors like hypoxia or increased erythropoiesis, which can attenuate the increase in hepcidin levels induced by IL-6. Nonetheless, the present study suggests that the definition of iron deficiency that is applied in heart failure is not universal and does not seem to be appropriate in maintenance HTx recipients.

We found that GDF-15 was elevated in the IronIC participants. In theory, the inhibitory effect of GDF-15 on hepcidin may have counteracted any IL-6-induced increase in hepcidin. However, the lack of correlation between hepcidin, GDF-15, and IL-6 suggests that the level of inflammation might not be sufficient to stimulate the synthesis of hepcidin in our HTx recipients. The restored ejection fraction, the reduced symptom burden and the immunosuppressive treatment initiated after HTx might also contribute to the modest level of inflammation and the normal hepcidin levels observed in the IronIC population. The choice of

immunosuppressant might also be important. Patients receiving mTOR treatment had more inflammation, lower transferrin saturation and higher sTfR than patients receiving CNI treatment.

Peak oxygen uptake increased in patients with ferritin below 30 $\mu\text{g/L}$ in the IronIC trial, and retrospective analyses show that peak VO_2 also improved in patients with sTfR above the reference range and in patients with sTfR/F > 2.1. In patients with heart failure, it is hypothesized that low hepcidin levels combined with elevated sTfR more precisely define iron deficiency.²⁵ Because different laboratories use different assays, the normal range for sTfR varies between laboratories. Consequently, there is no agreed-upon cut-point for sTfR or the sTfR/F index. We based our cut-point for the latter on the data at hand. The selected cut-off may therefore overestimate the discriminative value of the test. Nevertheless, our results suggest that HTx recipients with low hepcidin levels, low ferritin levels, or elevated sTfR might benefit from intravenous iron supplement.

It is important to assess the mechanisms of iron metabolism in HTx recipients and establish a suitable definition of iron deficiency to optimize post-transplant care. Iron deficiency can reduce exercise capacity and quality of life,^{26–28} and it independently predicts HTx and death in patients with heart failure,²⁹ although intervention studies with iron supplementation have not shown any effects on mortality. On the other hand, patients with iron overload cardiomyopathy may have inferior long-term survival after HTx compared with other HTx recipients.³⁰ In murine models, iron overload, as well as iron deficiency, is associated with accelerated graft rejection.³¹ Furthermore, iatrogenic iron overload increased cardiovascular morbidity and all-cause mortality in patients with end-stage renal disease.³² Moreover, iron may enhance oxidative stress and promote inflammation, underscoring the importance of using a strict definition of true iron deficiency before initiating iron supplementation.³³ When definitions of iron deficiency that have not been validated in HTx recipients are adapted to post-transplant management, the consequences may be iron overload or undetected iron deficiency, both of which should be avoided. Our results suggest that the optimal definition of iron deficiency in HTx recipients resembles the definition applied in healthy individuals. However, further investigation is required to establish which cut-offs that are suitable to define iron deficiency in the HTx population.

5 | LIMITATIONS

This is a retrospective analysis from a trial with a limited number of patients. The IronIC trial was designed to test the effect of intravenous iron in patients with iron deficiency as defined in heart failure. Patients with high levels of ferritin were therefore excluded from participation. The subgroup analyses were not prespecified and must be interpreted with caution. Patients who had received an allograft within the last year were excluded from participation in the IronIC trial. Consequently, we were not able to assess iron metabolism in newly transplanted patients. Because there is no established cut-point for the sTfR/F index, we

selected an optimal cut-off based on the material at hand. This cut-off is not validated, and the results must be regarded as hypothesis-generating only. Lastly, other factors than those assessed here might also be involved in the regulation of hepcidin and iron homeostasis.

6 | CONCLUSION

Our results suggest that iron metabolism is not substantially disturbed by inflammation in HTx recipients. Hepcidin levels were not elevated in our HTx recipients with ferritin <100 $\mu\text{g/L}$ or ferritin 100–300 $\mu\text{g/L}$ in combination with transferrin saturation of <20% although the inflammatory markers IL-6, PTX3, and CRP were increased compared with controls. However, hepcidin levels were suppressed in patients with very low ferritin and in patients with elevated sTfR. These patients benefited from intravenous iron supplement. The definition of iron deficiency used in heart failure may be too liberal to be used in HTx recipients, but ferritin, sTfR, and hepcidin may be used to select HTx recipients who might benefit from intravenous iron supplementation.

Central illustration

We examined circulating markers of iron metabolism in 97 heart transplant (HTx) recipients with iron deficiency defined as ferritin <100 $\mu\text{g/L}$ or ferritin 100–300 $\mu\text{g/L}$ and transferrin saturation <20%, and in 21 healthy control subjects. Baseline levels of hepcidin did not differ between HTx recipients and controls. The HTx recipients participated in a randomized placebo-controlled trial of intravenous ferric derisomaltose 20 mg/kg. Although the overall effect of iron on peak oxygen consumption was neutral, patients with ferritin <30 $\mu\text{g/L}$ or soluble transferrin receptor (sTfR) above normal benefitted from intravenous iron, as reflected by an increase in the change in oxygen consumption (ΔVO_2). The definition of iron deficiency used in heart failure may not be operational in HTx recipients, but patients with very low circulating levels of ferritin or elevated sTfR might benefit from intravenous iron supplementation.

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CONFLICTS OF INTEREST

Dr Broch has received lecture fees from Pharmacosmos, AstraZeneca, Boehringer Ingelheim, Pfizer, Orion Pharma, and Vifor Pharma and has sat on advisory boards for Pfizer and AstraZeneca. Dr Gullestad has received lecture fees from AstraZeneca, Boehringer Ingelheim, Novartis and Amgen and has sat on advisory board for AstraZeneca and Boehringer Ingelheim. The other authors do not have conflicts of interest pertaining to this work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author, Kaspar Broch. The data are not publicly available because they contain information that could compromise the privacy of research participants.

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