

Myocardial iron content and mitochondrial function in human heart failure: a direct tissue analysis

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Aims

Iron replacement improves clinical status in iron-deficient patients with heart failure (HF), but the pathophysiology is poorly understood. Iron is essential not only for erythropoiesis, but also for cellular bioenergetics. The impact of myocardial iron deficiency (MID) on mitochondrial function, measured directly in the failing human heart, is unknown.

Methods and results

Left ventricular samples were obtained from 91 consecutive HF patients undergoing transplantation and 38 HF-free organ donors (controls). Total myocardial iron content, mitochondrial respiration, citric acid cycle and respiratory chain enzyme activities, respiratory chain components (complex I–V), and protein content of reactive oxygen species (ROS)-protective enzymes were measured in tissue homogenates to quantify mitochondrial function. Myocardial iron content was lower in HF compared with controls (156 ± 41 vs. $200 \pm 38 \mu\text{g}\cdot\text{g}^{-1}$ dry weight, $P < 0.001$), independently of anaemia. MID (the lowest iron tercile in HF) was associated with more extensive coronary disease and less beta-blocker usage compared with non-MID HF patients. Compared with controls, HF patients displayed reduced myocardial oxygen₂ respiration and reduced activity of all examined mitochondrial enzymes (all $P < 0.001$). MID in HF was associated with preserved activity of respiratory chain enzymes but reduced activity of aconitase and citrate synthase (by -26% and -15% , $P < 0.05$) and reduced expression of catalase, glutathione peroxidase, and superoxide dismutase 2.

Conclusion

Myocardial iron content is decreased and mitochondrial functions are impaired in advanced HF. MID in HF is associated with diminished citric acid cycle enzyme activities and decreased ROS-protecting enzymes. MID may contribute to altered myocardial substrate use and to worsening of mitochondrial dysfunction that exists in HF.

Keywords

Heart failure • Mitochondria • Iron deficiency • Bioenergetics • Metabolism • Reactive oxygen species

Introduction

Cardiac mechanical function places high demand on adequate and timely provision of chemical energy by the mitochondria.¹ Mitochondrial dysfunction, characterized by decreased biogenesis, impaired substrate utilization, and increased reactive oxygen species (ROS) production is thought to contribute to cardiac

ageing and to the pathogenesis of heart failure (HF).^{2–5} Improved understanding of mitochondrial dysfunction associated with HF can provide novel therapies targeting myocardial energetics and efficiency.^{1,6}

Cellular energetic function is importantly modulated by iron.^{6–8} Mitochondrial enzymes involved in oxidative phosphorylation, antioxidative defence, and oxygen transport require iron in a ‘free’

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form, bound in haem or in Fe–S clusters. Iron availability also regulates mitochondrial biogenesis.^{9,10} While rare iron overload leads to ROS-mediated tissue toxicity and cardiomyopathy,¹¹ far more common iron deficiency also produces mitochondrial abnormalities in cardiac muscle.^{7,8} In HF, systemic iron deficiency is frequent,¹² and is associated with worse exercise capacity and increased mortality.^{13,14} Correction of anaemia through enhanced erythropoiesis does not help HF patients,¹⁴ but i.v. iron supplementation does improve exercise tolerance and quality of life, even in non-anaemic HF patients with systemic iron deficiency.^{15,16} This suggests that relative deficiency of iron in non-erythroid tissues might play an important role in the pathophysiology of HF.

Recent studies evaluating iron status in human HF have relied on circulating markers of the systemic iron level,^{12,14–17} but only very few examined directly myocardial iron content and revealed a poor relationship between myocardial and circulating iron markers.^{18,19} Little is known about iron content in the failing and non-failing human myocardium, its determinants, and about the consequences for myocardial mitochondrial function. To better understand these relationships, we sought to examine myocardial iron content and mitochondrial function directly in a large consecutive series of advanced HF patients and HF-free organ donors using LV tissue samples obtained at the time of transplantation.

Methods

For details and references, see the Supplementary material online, *Methods*.

Samples of failing LV myocardium were obtained from 91 consecutive patients undergoing transplantation for end-stage HF in IKEM (Prague, Czech Republic). Control samples were obtained from 38 consecutive organ donors without HF or systolic dysfunction, whose hearts were not used for transplantation for technical or medical reasons. Both in HF and in controls, the samples were obtained by punching the LV free wall, avoiding scarred areas and epicardial fat. Samples were placed without delay into liquid nitrogen and transported for long-term storage at -80°C . All HF subjects signed a written agreement (prior to the transplantation) for utilization of explanted tissue for research purposes. The protocol was approved by the Institutional Ethics Review Board. Myocardial iron content was determined by inductively coupled plasma mass spectrometry in lyophilized samples after digestion.^{20,21}

Mitochondrial function studies

Tissue homogenates were prepared from -80°C stored frozen samples of the left ventricle. Genomic DNA was isolated and quantitative real-time PCR of two mitochondrial target sequences (16S and D-loop) and one nuclear target (glyceraldehyde 3-phosphate dehydrogenase, GAPDH) was performed to quantify mitochondrial DNA (mtDNA) content, normalized to nuclear DNA (GAPDH gene). Succinate respiration, NADH respiration, and cytochrome *c* oxidase respiration were determined in homogenates using the high-resolution oxygraph.²² Spectrophotometric methods were used to measure enzyme activities of citrate synthase, aconitase, cytochrome *c* oxidase (COX, complex IV), succinate-cytochrome *c* reductase (SCCR, complexes II + III), and NADH-cytochrome *c* reductase (NCCR, complexes I + III).

Protein expression (western blotting)

Samples of myocardial homogenate were denatured, SDS–PAGE was performed, proteins were transferred to a membrane, and the following specific primary antibodies were used to assess expression of respiratory chain enzymes (NDUFA9 subunit of complex I, SDHA subunit of complex II, Core2 subunit of complex III, Cox4 subunit of complex IV, $F_1\text{-}\alpha$ subunit of complex V), transferrin receptor, antioxidative enzymes [superoxide dismutase (SOD) 1 and 2, catalase, glutathione peroxidase, glutathione reductase], and markers of cellular (pan-actin), cardiomyocyte (sarcomeric α -actin, calsequestrin), and mitochondrial content (porin). For quantitative detection, the corresponding infrared fluorescent secondary antibodies were used and the signal was quantified using an infrared scanner and image analyser. The expression of mitochondrial proteins (respiratory chain components I–V, SOD2) was normalized to the expression of porin.

Statistical analysis

Data are expressed as means \pm SD. The JMP10 (SAS, Cary, NC, USA) statistical package was used. Distribution normality was assessed with Shapiro–Wilk test. Groups were compared using unpaired *t*-test or χ^2 test. A general linear model with one or more variables was used for standard least square regression analysis. Correlation analysis was performed using Pearson's test. A *P*-value of <0.05 was considered significant.

Results

Compared with controls, HF patients displayed severe LV dysfunction, were slightly older, more anaemic, and more often males (Table 1). Causes of death in controls included haemorrhagic stroke (56%), head trauma (26%), post-hypoxic brain oedema (14%), and other causes in 5%.

Myocardial iron content in heart failure

Compared with controls, HF patients had lower total myocardial iron with leftward shift of the distribution curve (156 ± 41 vs. $200 \pm 38 \mu\text{g}\cdot\text{g}^{-1}$ dry weight, $P < 0.001$, Figure 1). Myocardial iron deficiency (MID) was defined *a priori* by myocardial iron content $<142.9 \mu\text{g}\cdot\text{g}^{-1}$ dry weight, corresponding to the cut-off value for the first tercile of the iron distribution in the HF group. Non-MID HF patients displayed lower myocardial iron content than controls (Figure 1B). The patients in the low decile of myocardial iron distribution had markedly increased expression of transferrin receptor, confirming the presence of an iron-deficient state (Figure 1C).

Heart failure patients with MID had more severe CAD (by number of diseased vessels), and lower doses of beta-blockers or renin–angiotensin system inhibitors than non-MID HF subjects (Table 1). HF patients with MID had similar severity of LV dysfunction and haemodynamic impairment as non-MID patients (Table 2). Myocardial iron content correlated weakly with haemoglobin concentration in HF ($r = 0.21$, $P = 0.05$, Figure 2), but not in controls ($r = -0.1$, $P = 0.6$), and was not associated with inflammation or renal insufficiency in HF.

In HF patients and controls, myocardial samples displayed similar expression of pan-actin, indicating similar cellularity, but reduced

Table 1 Baseline characteristics

	Controls (n = 38)	HF (n = 91)	P-value	HF no MID (n = 61)	HF with MID (n = 30)	P-value
Age, years	42 ± 15	53 ± 11	<0.001	52 ± 11	55 ± 10	0.2
Male gender	50%	83%	<0.001	89%	73%	0.07
Body mass index, kg/m ²	24 ± 5.7	26 ± 4.7	0.1	26 ± 5	27 ± 4	0.3
Ischaemic aetiology of HF	–	46%	–	40%	55%	0.2
CAD severity (0–3) ^a	–	1.3 ± 0.1	–	1.3 ± 1.3	1.9 ± 1.4	0.04
HF duration, years	–	7.4 ± 7.14	–	6.8 ± 6.0	9.1 ± 9.3	0.2
History of coronary artery bypass grafting, %	–	14%	–	10%	24%	0.08
Previous left ventricular assist device, %	–	9%	–	10%	7%	0.6
NYHA grade, I–IV	–	3.3 ± 0.6	–	3.3 ± 0.6	3.3 ± 0.6	0.8
BNP, ng/L	–	1411 ± 1277	–	1385 ± 1181	1562 ± 1411	0.6
Anaemia	14%	51%	0.003	45%	63%	0.09
Diabetes mellitus	–	24%	–	25%	30%	0.6
Furosemide dose, mg/day	–	121 ± 98	–	117 ± 78	128 ± 129	0.7
Beta-blocker dose, 0–3 ^b	–	1.0 ± 0.7	–	1.1 ± 0.8	0.7 ± 0.6	0.005
ACE inhibitor/ARB dose, 0–3 ^b	–	0.7 ± 0.7	–	0.7 ± 0.7	0.5 ± 0.6	0.04
Haemoglobin, g/L ^c	139 ± 11	128 ± 23	0.002	132 ± 21	119 ± 23	0.01
Mean red cell volume, fL	–	90 ± 5.6	–	91 ± 7	90 ± 6	0.3
Leucocytes, 10 ⁹ /L	–	8.0 ± 2.7	–	8.0 ± 2.4	8.0 ± 3.2	0.9
C-reactive protein, mg/L	–	15 ± 31	–	11 ± 18	25 ± 50	0.2
eGFR, mL/min/1.73 m ²	94 ± 39	70 ± 28	<0.001	71 ± 29	64 ± 23	0.3
Haemoglobin A _{1c} , %	–	4.7 ± 0.8	–	4.7 ± 0.7	4.7 ± 0.9	0.9

Values are means ± SD.

eGFR, estimated glomerular filtration rate; calculated by 2009 CKD-EPI equation; HF, heart failure; MID, myocardial iron deficiency.

^aNumber of major epicardial vessels affected by significant (≥75%) stenosis.

^bDrug dose/day (3 = high, i.e. recommended target dose; 2 = between 1 and 3; 1 = low dose, i.e. ≤25% of target dose; 0, not administered).

^cThe admission haemoglobin level was available from 21 non-trauma controls.

expression of sarcomeric α -actin and calsequestrin in HF, indicating lower sarcomere density (Table 3). The expression of the outer membrane mitochondrial protein porin (a marker of mitochondrial mass) was similar in controls and HF patients, regardless of iron status, indicating that tissue composition cannot explain variation of myocardial iron in HF. Despite similar porin expression, HF-MID patients had less mtDNA than non-MID patients, suggestive of similar mitochondrial abundance, but altered mitochondrial composition. In all subjects, mtDNA correlated with iron content (16S, $r = 0.21$, $P = 0.04$; D-loop, $r = 0.22$, $P = 0.01$). An analysis in an age- and gender-matched subset of controls and subjects with HF ($n = 28/n = 73$, Supplementary material online, Table S1) showed reduced myocardial iron content and mitochondrial dysfunction in HF, indicating that between-group differences cannot be explained by gender or age imbalance in our study cohorts.

Mitochondrial function in heart failure and myocardial iron deficiency

Activities of all mitochondrial enzymes examined, i.e. aconitase, citrate synthase, NCCR, SCCR, and COX, were significantly reduced in HF compared with controls (–19, –22, –27, –27, and –33%, all $P < 0.001$, Figure 3). The presence of MID in HF was associated with further reduction of activities of citric acid cycle enzymes

(aconitase and citrate synthase by –26% and –15%, $P < 0.05$, Figure 3A). The activities of respiratory chain oxidoreductases (COX, SCCR, and NCCR) were similar to those in non-MID HF patients (Figure 3B). Mitochondrial oxygen₂ consumption (NADH-, succinate-, or COX-dependent respiration), which reflects respiratory chain activity, was significantly reduced in HF compared with controls (by –28, –22, and –15%, Table 4), but MID had no impact on respiration.

At the level of protein expression (based on quantification of representative subunits), patients with HF displayed significant reduction of respiratory chain complex I (by –22%, $P < 0.001$) and complex III (by –15%, $P = 0.03$) compared with controls (Figure 4A), with no difference between MID and non-MID HF patients. Correlation analysis in HF subjects found a positive correlation between myocardial iron content and the enzymatic activities of aconitase, citrate synthase (Figure 3A), and SCCR (Table 5; Figure 3B).

Reactive oxygen species defence in heart failure and myocardial iron deficiency

Among the ROS-protective enzymes examined, the expression of glutathione reductase was significantly reduced in HF (by –17%) compared with controls (Figure 4B). The presence of MID in HF was associated with significant reduction of catalase (by –23%), SOD2

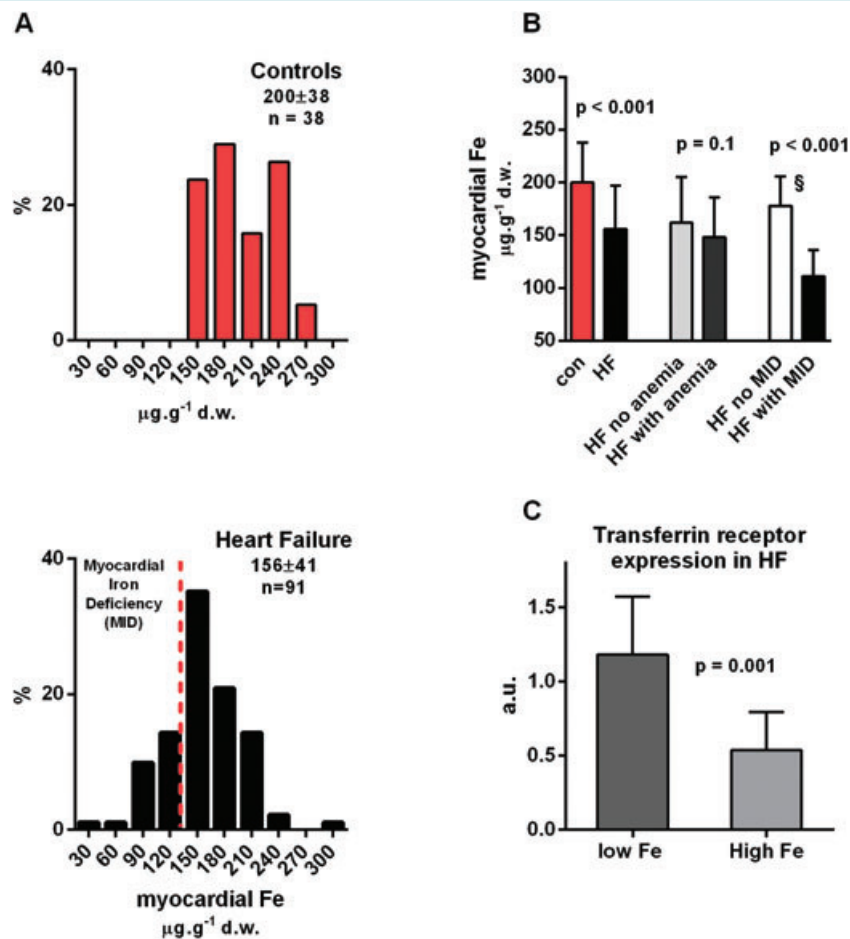


Figure 1 Myocardial iron content. (A) The distribution of myocardial iron content [$\mu\text{g}\cdot\text{g}^{-1}$ of dry weight (d.w.)] in controls and heart failure (HF) patients. Iron levels (mean \pm SD) by groups. $\S P < 0.001$ vs controls. (B) The interrupted line depicts the arbitrary threshold of myocardial iron deficiency (MID; $142.9 \mu\text{g}\cdot\text{g}^{-1}$ d.w.), defined as the lower tercile cut-off of the HF group. (C) Transferrin receptor expression (western blot) in the top and low decile of myocardial iron distribution in HF patients.

(by -20%), and glutathione peroxidase (by -21%), as compared with non-MID HF. In all HF subjects, myocardial iron content significantly correlated with the expression of catalase, glutathione peroxidase, and glutathione reductase (Table 5).

The impact of anaemia on mitochondrial function

Anaemia was present in 51% of HF patients. Anaemic HF patients had more CAD, fewer HF medications, and more renal dysfunction and inflammation compared with non-anaemic HF patients (Supplementary material online, Table S2). Anaemic HF patients also displayed higher right atrial pressure compared with non-anaemic HF patients. In contrast to MID, anaemia itself had no associations with myocardial mitochondrial function as shown by direct comparison and bivariate regression analysis (Supplementary material online, Tables S2 and S3).

Discussion

This is the first human study that systematically and directly examined associations between myocardial iron content and mitochondrial function in tissues taken from patients with and without HF. Myocardial iron content was 22% lower in patients with HF compared with controls. MID was not predicted by the presence of anaemia, indicating that systemic and myocardial iron contents are differentially regulated. Mitochondrial function was profoundly impaired in patients with HF, manifested by lower respiratory chain protein expression, decreased enzymatic activities, and lower oxygen consumption compared with controls. HF patients with MID had preserved mitochondrial respiration, but diminished activities of key citric acid cycle enzymes and decreased expression of ROS-protecting enzymes, indicating that MID may contribute to worsening of mitochondrial dysfunction that exists in HF by altering intermediary substrate metabolism and ROS handling. The results suggest that restoring myocardial iron content may therefore improve substrate utilization and myocardial bioenergetics.

Table 2 Cardiac function: echocardiography, and right heart catheterization

	Controls (n = 38)	HF (n = 91)	P-value	HF no MID (n = 61)	HF with MID (n = 30)	P-value
LV ejection fraction, %	57 ± 8	23 ± 8	<0.001	22 ± 8	23 ± 7	0.8
LV end-diastolic dimension, mm	46 ± 5	69 ± 11	<0.001	70 ± 11	69 ± 9	0.7
LV mass/BSA, g/m ²	90 ± 27	138 ± 36	<0.001	142 ± 38	129 ± 29	0.09
Systolic blood pressure, mmHg	118 ± 23	106 ± 14	0.003	106 ± 13	109 ± 17	0.4
Diastolic blood pressure, mmHg	70 ± 14	80 ± 13	0.3	73 ± 9	74 ± 10	0.8
Heart rate, b.p.m.	91 ± 24	80 ± 13	0.01	80 ± 13	79 ± 11	0.7
Cardiac index, L/min/m ²	–	2.0 ± 0.5	–	2.0 ± 0.5	2.0 ± 0.6	0.9
Mean PA pressure, mmHg	–	32 ± 11	–	31 ± 11	35 ± 11	0.1
PA wedge pressure, mmHg	–	23 ± 9	–	22 ± 9	25 ± 9	0.1
Right atrial pressure, mmHg	–	9 ± 5	–	9 ± 5	10 ± 6	0.3

Values are means ± SD.

BSA, body surface area; HF, heart failure; MID, myocardial iron deficiency; PA, pulmonary artery.

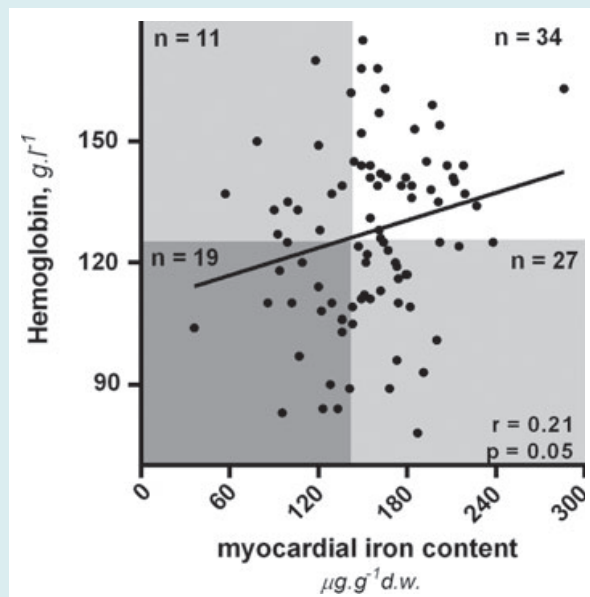


Figure 2 Relationship between anaemia and myocardial iron content. The graph illustrates the weak relationship between myocardial and systemic iron status reflected by haemoglobin concentration. Shaded areas indicate patients with myocardial iron deficiency or anaemia (for definitions, see the Methods). r = Pearson's correlation coefficient.

Mitochondrial function in heart failure and the impact of myocardial iron deficiency

Heart failure is often associated with mitochondrial impairment that may further promote cardiomyocyte dysfunction.^{1,6} Consistent with this idea, HF patients in our study displayed profound reduction of mitochondrial respiration, and reduced citric acid cycle and respiratory chain enzymatic activities, congruent with previous studies in human failing myocardium.^{3,5,23–25} We

observed that these alterations were not influenced by the primary HF aetiology or co-morbidities, indicating that mitochondrial dysfunction represents a final common pathway for several types of myocardial injury.

A key observation of our study is that a part of mitochondrial alterations in HF may be related to mitochondrial iron deficit that may worsen HF-related mitochondrial impairment. It is a plausible idea, because multiple mitochondrial enzymes involved in oxidative phosphorylation or antioxidative defence use iron as a metal cofactor, either bound in haem or in Fe–S clusters.^{14,26} Additionally, cellular iron availability in muscle cells regulates mitochondrial gene expression in comparable magnitude to, but independently of, the established drivers of mitochondrial biogenesis, such as peroxisome proliferator-activated receptor- γ coactivators (PGC-1 α and β).⁹ The causal link between reduced cellular iron content and mitochondrial dysfunction in HF is supported by a recent observation that cardiac-specific inactivation of transferrin receptor 1, a model of isolated cardiac iron deficiency, leads to severe myocardial iron depletion, failure of mitochondrial respiration, and lethal cardiomyopathy, that is partly rescued by iron administration.¹⁰

In our study, HF patients with MID displayed preserved activity of respiratory chain enzymes but reduced activity of citrate synthase and aconitase, key enzymes of the citric acid cycle (Figure 3). Previous *in vitro* experiments in cardiomyocytes demonstrated that iron deficiency has negative effects on activity of these two enzymes.^{27,28} Diminished citrate synthase and aconitase activity due to MID may translate into lower flux through the citric acid cycle and impaired energetic reserve of cardiomyocytes.²⁶ Lower citric acid cycle flux may slow down utilization of acetyl-CoA generated by fatty acid oxidation, while preserving glucose utilization via glycolysis, as was already observed under iron-deficient experimental conditions.^{10,27} Myocardial iron deficit may therefore contribute to the shift from fatty acids to glucose utilization that is typical for the failing heart.^{1,6}

Table 3 Tissue composition and mitochondrial content

	Controls (n = 38)	HF (n = 91)	P-value	HF no MID (n = 61)	HF with MID (n = 30)	P-value
Cellular content						
Pan-actin, a.u.	1.01 ± 0.37	1.02 ± 0.29	0.9	1.04 ± 0.30	0.97 ± 0.30	0.4
Cardiomyocyte content						
α-Actinin sarcomeric, a.u.	1.13 ± 0.33	0.94 ± 0.20	0.006	0.96 ± 0.21	0.86 ± 0.14	0.05
Calsequestrin, a.u.	1.11 ± 0.35	0.95 ± 0.26	0.03	0.98 ± 0.27	0.86 ± 0.25	0.1
Mitochondrial content						
Porin, a.u.	1.04 ± 0.21	0.99 ± 0.25	0.3	1.00 ± 0.24	0.95 ± 0.27	0.3
mtDNA (16S), 2 ^{-ΔCt}	8316 ± 2817	7760 ± 2896	0.3	8218 ± 3140	6843 ± 2086	0.01
mtDNA (D-loop), 2 ^{-ΔCt}	4681 ± 1723	4197 ± 1520	0.1	4385 ± 1667	3820 ± 1101	0.06

Values are means ± SD.

a.u., arbitrary units; HF, heart failure; MID, myocardial iron deficiency; mtDNA, mitochondrial DNA.

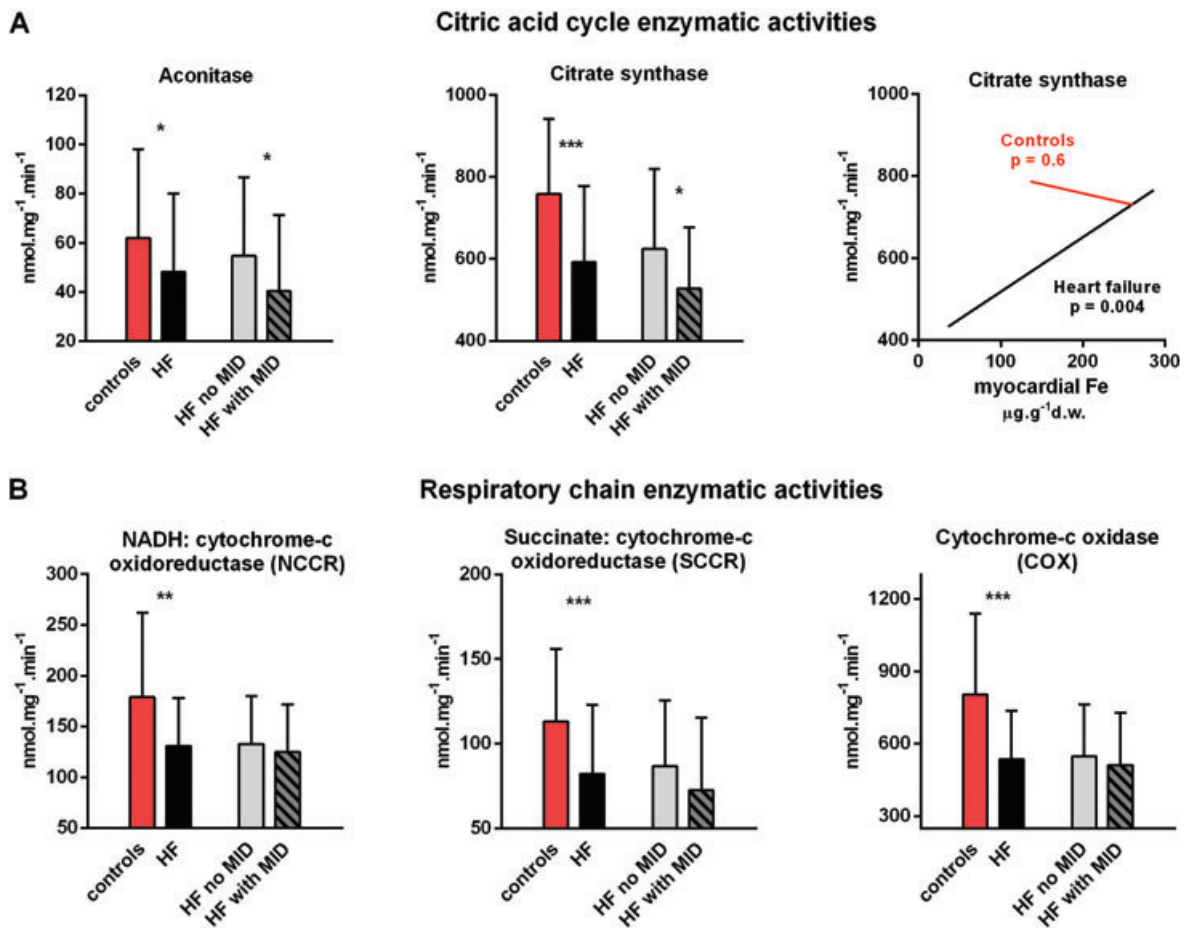


Figure 3 Mitochondrial enzymatic activities. (A) Activity of citric acid cycle enzymes of aconitase and citrate synthase by groups and the correlation between citrate synthase activity and myocardial iron. (B) Activity of respiratory chain enzymes by groups. d.w., dry weight; HF, heart failure; MID, myocardial iron deficiency. * $P \leq 0.05$, ** $P \leq 0.001$, *** $P \leq 0.0001$ by *t*-test. Bars represent the mean ± SD.

Table 4 Mitochondrial respiration

	Controls (n = 38)	HF (n = 91)	P-value	HF no MID (n = 61)	HF with MID (n = 30)	P-value
NADH respiration, pmol O ₂ /s/mg	1091 ± 502	785 ± 379	0.003	811 ± 369	730 ± 402	0.4
Succinate respiration, pmol O ₂ /s/mg	653 ± 244	508 ± 211	0.004	537 ± 224	454 ± 175	0.06
COX respiration, pmol O ₂ /s/mg	1895 ± 773	1602 ± 652	0.05	1615 ± 626	1576 ± 711	0.8

Values are means ± SD.
COX, cytochrome c oxidase; HF, heart failure; MID, myocardial iron deficiency.

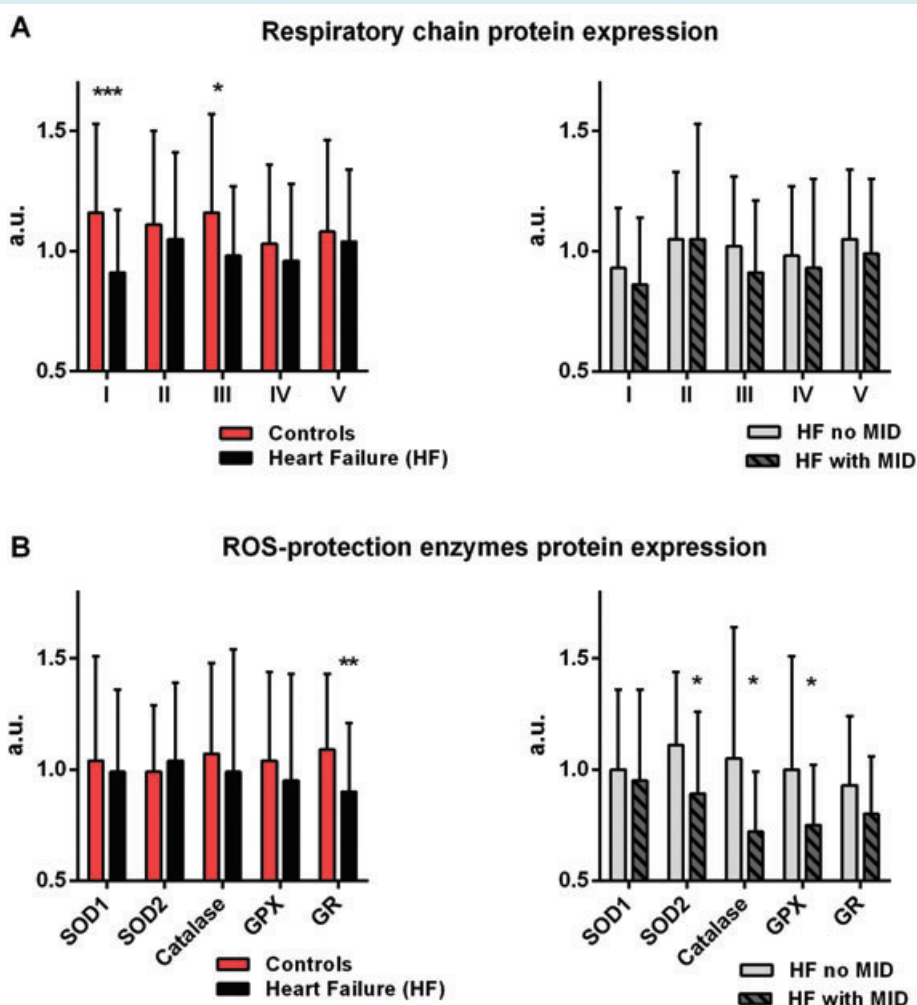


Figure 4 Respiratory chain and reactive oxygen species (ROS) defence protein expression. (A) Expression of respiratory chain components (complex I–V). (B) Expression of ROS-protective enzymes: superoxide dismutase (SOD), glutathione peroxidase (GPX), and glutathione reductase (GR). Expression of mitochondrial proteins (complex I–V, SOD2) was normalized to the outer mitochondrial membrane protein porin. a.u., arbitrary units; HF, heart failure; MID, myocardial iron deficiency. **P* ≤ 0.05, ***P* ≤ 0.001, ****P* ≤ 0.0001 by *t*-test. Bars represent the mean ± SD.

Table 5 Correlation analysis between myocardial iron content and myocardial mitochondrial functions in heart failure patients

	<i>r</i>	<i>P</i> -value
Mitochondrial and tissue composition		
Pan-actin	0.09	0.4
Sarcomeric actin	0.13	0.3
Casequestrin	0.15	0.3
Porin	−0.02	0.9
mtDNA 16S/mtDNA D-loop	0.22/0.17	0.04/0.1
Enzymatic activities		
Aconitase	0.23	0.03
Citrate synthase	0.30	0.004
NADH:cytochrome c oxidoreductase	0.1	0.3
Succinate:cytochrome c oxidoreductase	0.24	0.02
COX	0.18	0.08
Respiration		
NADH respiration	0.18	0.09
Succinate respiration	0.20	0.07
COX respiration	0.10	0.3
Protein expression		
colI/colII/colIII/colIV/coV	0.16/0.09/0.16/ 0.08/0.16	0.1/0.4/0.1/ 0.5/0.1
SOD1/SOD2	0.06/0.13	0.7/0.3
Catalase	0.30	0.01
GPX/GR	0.29/0.27	0.02/0.02

r = Pearson's correlation coefficient.

co, complex; COX, cytochrome c oxidase; mtDNA, mitochondrial DNA; SOD, superoxide dismutase; GPX, glutathione peroxidase; GR, glutathione reductase.

A complementary adverse impact of MID may be related to handling of ROS.²⁹ Experimental nutritionally induced iron deficiency is associated with increased ROS production in the heart.^{7,29} HF patients with MID displayed reduced expression of mitochondrial SOD2, catalase, and glutathione peroxidase. In the whole HF group, we observed a correlation between myocardial iron content, glutathione peroxidase, glutathione reductase, and catalase, an iron-containing enzyme previously implicated in the transition from heart hypertrophy to failure.² The major source of intracellular ROS is the respiratory chain—an impaired enzymatic activity of complex I couples directly with enhanced superoxide production in HF.⁴ In an iron-deficient HF state, an enhanced ROS production in HF may couple with reduced ROS protection, which may promote contractile dysfunction and maladaptive remodelling. Further experiments and direct ROS measurements are, however, needed to confirm this notion.

Determinants of myocardial iron content in heart failure

Previous smaller studies in patients with HF reported a reduction of myocardial iron in the range of 16–29% compared with controls,^{18,19} which is in line with our observation. The reason for lower myocardial iron content in HF is unknown. We found only

marginal correlations between myocardial iron and haemoglobin concentration, which confirms that MID can be encountered even in non-anaemic HF patients.^{14,19} In addition, co-morbidities typically associated with anaemia in HF,¹⁴ such as inflammation or renal insufficiency, were also not associated with MID in our cohort. Previous studies also noted only weak correlations of myocardial iron content with circulating markers of systemic iron status,^{18,19} suggesting that systemic and myocardial iron content are partly independent and the one does not simply reflect the other.

Myocardial iron content was inversely related to severity of CAD. This may speak for an influence of ischaemia on local myocardial factors responsible for iron transport and utilization (ferroportin, transferrin, or hepcidin), that may be differentially regulated in ischaemic³⁰ or failing³¹ myocardium. Sympathetic nervous system hyperactivation may also play a role, as HF patients with MID had less beta-blockers than non-MID HF patients. *In vitro*, cardiomyocytes exposed to norepinephrine down-regulate transferrin receptor, the gateway for the entry of iron into the cell, providing a possible link between neurohumoral activation, diminished iron uptake, and MID in HF.¹⁸

The present study has several important limitations. Owing to the cross-sectional design, causality cannot be gleaned from our data, although recent experimental studies support the mechanistic link between myocardial iron depletion and mitochondrial and cardiac dysfunction.^{9,10} The groups of HF patients with or without MID were different in their underlying heart disease (the severity of CAD), in drug therapy, or in the degree of anaemia. These variables must be acknowledged as potential confounding factors that may contribute to the observed differences in mitochondrial function. We studied HF patients who were selected for transplantation, so co-morbidities were relatively rare and patients with potential iron overload from chronic liver disease (including haemochromatosis) or alcohol abuse were excluded. We did not measure systemic circulating iron biomarkers, but it is already known that they poorly predict myocardial iron content.¹⁹ Owing to logistic restraints, we used tissue homogenates from flash-frozen myocardial tissue. This approach, extensively validated in our lab,²² enables us to process adequate numbers of patient samples, but it does not allow the same spectrum of examinations as freshly isolated mitochondria. We measured only total tissue iron, ignoring differences between non-haem iron and haem iron that might be increased in HF.³² In our experience, frozen tissue does not allow reliable isolation of intact mitochondria, so we did not address subcellular compartmentalization of iron (mitochondrial vs. cytoplasmic).

In conclusion, we demonstrated that mitochondrial function is profoundly impaired in HF patients compared with controls and that myocardial iron deficit, common in advanced HF, may worsen mitochondrial metabolism and ROS handling. These relationships may lead to reduced substrate flexibility and impaired energy production in iron-deficient failing myocardium. Our results suggest that restoring normal myocardial iron levels may help to improve bioenergetics of the failing heart.

Supplementary Information

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

Table S1. Comparison of age- and gender-matched subgroup of HF patients and control subjects.

Table S2. The clinical correlates of anaemia in HF.

Table S3. The impact of anaemia and myocardial iron content on mitochondrial function using a general linear model.

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Conflict of interest: none declared.

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