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Myocardial and Systemic Iron Depletion in Heart Failure

Implications for Anemia Accompanying Heart Failure

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Objectives This study sought to determine the potential pathophysiological link between anemia and disease severity, and adverse outcome in heart failure (HF).

Background Anemia frequently accompanies advanced HF; however, the pathophysiological mechanism responsible for the association between anemia and more severe HF remains uncertain. We hypothesized that a depletion of myocardial iron content may provide the biological link.

Methods Complementary clinical and basic studies were performed. Hemodynamic, biochemical, and echocardiographic investigations were performed in 9 healthy controls and 25 patients with advanced HF (left ventricular ejection fraction: 23 ± 10%). Tissue iron content and type 1 transferrin receptor (Tfr1) expression were assessed in human myocardial tissue, and the regulation of Tfr1 expression was studied in isolated cardiomyocytes.

Results HF patients displayed evidence of iron deficiency as measured by lower serum iron ($p < 0.05$) and transferrin saturation (TFS) ($p < 0.05$). When subclassified according to the presence of anemia, TFS was lower in anemic compared with nonanemic HF patients, whereas TFS in nonanemic HF patients was intermediate. In association, myocardial iron content was reduced in HF versus non-HF samples (0.49 ± 0.07 μg/g vs. 0.58 ± 0.09 μg/g, $p < 0.05$), and there was a significant reduction ($p < 0.05$) in the myocardial mRNA expression of Tfr1, which plays a key role in cellular iron transport. In the context of HF, catecholamines and aldosterone both downregulated Tfr1 expression in isolated cardiomyocytes.

Conclusions This study suggests the presence of iron depletion in the failing human heart, providing a potential link for the association between anemia and adverse prognosis in HF. (J Am Coll Cardiol 2011;58:474–80) © 2011 by the American College of Cardiology Foundation

Anemia is a common comorbid factor in patients with heart failure (HF) (1) and is characteristically associated with greater functional impairment and worse New York Heart Association functional class (2). Recently, particular interest has been directed toward the strong inverse relationship between the extent of anemia and survival (3–8). This observation has subsequently been the logical basis of many trials aimed at evaluating the hypotheses that reversal of anemia may translate into improved survival for HF patients. Among these studies, several have evaluated the effect of the administration of erythropoietin and other erythropoiesis-stimulating agents, with limited effect (9).

Despite the focus on anemia as a target for therapy in HF, the cause of anemia in HF patients is a matter of ongoing debate. Similarly, the mechanism by which the presence of anemia contributes to an adverse outcome in these patients is also unknown. Specifically, it is not known whether it is the anemia per se that contributes to adverse prognosis or whether it is one of the contributing factors to the anemia that also contributes to adverse HF outcome or, alternatively, whether anemia is simply a biomarker for advanced HF.

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In regard to the etiology of the anemia in HF, there is growing evidence that iron availability may be reduced (1), and recently, it has been suggested that iron deficiency itself may be an independent predictor of outcome in HF (8). Conceptually, this may result from either an absolute

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deficiency of iron due to decreased enteral iron absorption and/or occult bleeding (10). Alternatively, iron deficiency may be relative, the result of dysregulation of iron homeostasis and accumulation of iron in cells of the reticuloendothelial system (11), which is a characteristic feature of anemia of chronic disease (12). In support of the important role of iron deficiency in HF patients, a recent trial of iron supplementation demonstrated an improvement in functional capacity and quality of life parameters (13).

In the present study, we aimed to test the hypothesis that the association between anemia and HF severity, and outcome might rather be explained by an underlying depletion of iron stores, particularly at the myocardial level. This concept is predicated on previous experimental work showing that iron deficiency is associated with progressive left ventricular dysfunction and cardiac fibrosis (14), although it is not known whether a similar entity exists in humans. In the context of HF, systemic and myocardial iron deficiency could thus contribute both to anemia and HF progression. In order to address this question, we performed a series of complementary studies in controls and HF patients, including biochemical and hemodynamic assessments and transcardiac blood sampling. These studies were complemented by determining tissue iron content and transferrin receptor expression in human heart samples, and by investigating the regulation of the transferrin receptor in cardiomyocyte cell culture.

Methods

Patients and protocol. The clinical protocol was approved by the Alfred Hospital Ethics Review Committee. We studied 25 unselected consecutive patients (18 males and 7 females) with advanced HF and significantly impaired left ventricular ejection fraction (LVEF) (<35%), referred by their managing physicians for hemodynamic assessment as a component of a standardized inpatient evaluation for heart transplantation. The cause of HF was ischemic heart disease in 9 patients and nonischemic cardiomyopathy in 16 individuals. Medications included angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (80%; median dose 50% [interquartile range (IQR): 25% to 50%] of target dose), beta-blockers (84%; median 50% [IQR: 25% to 50%] of target dose), loop diuretics (100%; median 240 mg [IQR: 40 to 268 mg] furosemide), and spironolacatone (80%; median 25 mg [25 to 50 mg]), and 84% had an implantable defibrillator. Patients were included irrespective of their hemoglobin (Hb) concentrations, and those on intravenous inotropes, severe renal impairment (estimated glomerular filtration rate [eGFR]: <30 ml/min/1.73 m^2), acute or chronic infection, known neoplasms, diseases of the gastrointestinal tract, overt thyroid disease, and patients currently treated with iron or erythropoiesis-stimulating agents or treated with iron or erythropoiesis-stimulating agents during the 3 months preceding the study were excluded. In conjunction, we also studied a group of healthy volunteer subjects (n = 9, 6 males and 3 females) recruited from the general community by advertisement.

Abbreviations and Acronyms

eGFR = estimated glomerular filtration rate
Hb = hemoglobin
HF = heart failure
hs-CRP = high-sensitivity C-reactive protein
IQR = interquartile range
LVEF = left ventricular ejection fraction
PCR = polymerase chain reaction
Tfr1 = type 1 transferrin receptor
TFS = transferrin saturation

Cardiac catheterization. Cardiac catheterization was performed in the nonfasting state and under full medication as appropriate. A 3-F arterial line was placed in a radial or brachial artery for blood pressure measurement and blood sampling. A coronary sinus catheter was inserted via an introducer sheath placed in the right internal jugular or brachial vein and positioned under fluoroscopic control. Right heart pressures and cardiac output were subsequently measured by Swan-Ganz catheterization.

Laboratory analysis. Hemoglobin, vitamin B_{12}, folate, creatinine, urea, high-sensitivity C-reactive protein (hs-CRP), and N-terminal-pro-B-type natriuretic peptide were measured in peripheral venous blood using standard commercial assays. Anemia was defined as Hb <13.0 g/dl in men or <12.0 g/dl in women (1). Iron, transferrin, soluble transferrin receptor, and ferritin levels were measured in arterial, coronary sinus, and peripheral venous blood using standard clinical assays. Transferrin saturation (TFS) was calculated from iron and transferrin (15). Transcardiac gradients and whole-body arteriovenous differences in various biochemical indexes were calculated and are presented in the subsequent text. All analyses were performed in the clinical laboratory of the Alfred Hospital.

Human myocardial tissue iron content and transferrin receptor expression. Myocardial tissue samples were obtained from the explanted failing hearts of a separate cohort of patients at the time of transplantation (n = 6, 5 males, age 46 ± 12 years) and from unused donor hearts (n = 5, 5 males, age 38 ± 18 years, p = NS vs. HF samples). Myocardial iron content was determined by atomic adsorption spectroscopy (Regional Laboratory Services, Benalla, Australia). Total RNA was extracted using Trizol (Invitrogen, Carlsbad, California). Following reverse transcription, real-time polymerase chain reaction (PCR) was performed using a PCR 7300 (Applied Biosystems, Foster City, California) thermocycler to investigate the expression of the type 1 transferrin receptor (Tfr1) using the primer pairs: (rat) forward: 5′-GAATAC GTTCCCCGTTGTTGA-3′, reverse: 5′-ATCCCCAGTTCCTAGATGAGCAT-3′ or (human) forward: 5′-GTGACCCTTACACAGCTGG-ATTC-3′, reverse: 5′-TGATGACCGAGATGGTGG-3′ together with the housekeeping genes 18S and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as appropriate. Quantitative data are expressed in ΔΔCt format.

Transferrin receptor expression in cardiomyocytes. Neonatal rat cardiomyocytes and cardiac fibroblasts were iso-

lated from D1-2 Sprague Dawley rat pups. The effect of exposure to neurohormones of relevance to HF on transferrin receptor expression was examined by real-time PCR as described in the previous text in n = 4 separate experiments.

Statistical analysis. Categorical data are given as counts and percentages, and continuous data are presented as mean ± SD or median (interquartile range), as appropriate. Between-group comparisons were performed using chi-square tests, unpaired *t* tests, or Mann-Whitney *U* tests, as appropriate. Comparisons between multiple groups were performed using 1-way analysis of variance or Kruskal-Wallis tests followed by Tukey tests or Mann-Whitney test, respectively, the latter with the p value adjusted for multiple testing (Bonferroni correction). Correlations between parameters of interest were assessed using Pearson correlation coefficients after ln-transformation of data with skewed distribution. Analysis was performed using commercially available software packages (SPSS, version 15.0, SPSS, Inc., Chicago, Illinois, and SigmaPlot, version 10.0, Systat Software Inc., San Jose, California).

Results

Clinical, laboratory, and hemodynamic features. As expected, controls and HF patients differed significantly with regard to LVEF (66 ± 9% vs. 23 ± 10%, $p < 0.01$), N-terminal-pro-B-type natriuretic peptide (53 ng/l [IQR: 23 to 90 ng/l] vs. 2,654 ng/l [IQR: 1,313 to 5,926 ng/l], $p < 0.01$), eGFR (90 ± 24 ml/min/1.73 m^2 vs. 70 ± 20 ml/min/1.73 m^2, $p < 0.05$), Hb (140 ± 9 g/l vs. 124 ± 20 g/l, $p < 0.05$), but did not differ in regard to body mass index. In regard to hematinic status, controls and HF patients differed significantly with regard to serum iron (18.4 ± 4.8 μmol/l vs. 12.3 ± 7.0 μmol/l, $p < 0.05$), serum transferrin (2.47 g/l [IQR: 2.20 to 2.60 g/l] vs. 2.84 g/l [IQR: 2.58 to 3.13 g/l], $p < 0.05$), and TFS (30.3 ± 10.2% vs. 17.3 ± 9.5%, $p < 0.05$), whereas serum ferritin did not differ (100 ± 57 μg/l vs. 124 ± 90 μg/l, p = NS).

In order to investigate the potential basis for the adverse association of anemia with HF severity, patients were stratified into the presence or absence of anemia defined by the criteria in the previous text. As shown in Table 1, HF patients with or without anemia did not differ significantly with regard to their hemodynamic profile at rest or with respect to renal function. Anemic HF patients displayed evidence of iron deficiency as reflected by lower serum iron and lower TFS as compared with both healthy controls and nonanemic HF patients. In conjunction, TFS was also lower in the nonanemic HF patients as compared with healthy controls (Fig. 1). Anemic HF patients as compared with nonanemic HF patients did not significantly differ with regard to their use and doses of angiotensin-converting enzyme inhibitors/angiotensin receptor blockers (78% vs. 82%, p = 0.84; 25% [IQR: 25% to 27%] vs. 50% [IQR: 25% to 67%] of target dose, p = 0.06), beta-blockers (67% vs. 94%, p = 0.08; 50% [IQR: 48% to 108%] vs. 50% [IQR: 25% to 50%] of target dose, p = 0.14), loop diuretics (100% in both groups, 160 mg [IQR: 80 to 310 mg] vs. 240 mg [IQR: 73 to 290 mg] furosemide, p = 0.71), and spironolactone (78% vs. 88%, p = 0.92; 25 mg [IQR: 25 to 25 mg] vs. 25 mg [IQR: 25 to 50 mg], p = 0.44), or device therapy (data not shown).

There was a very strong ($r^2 = 0.70$, $p < 0.001$) association between TFS and Hb across the entire cohort (Fig. 1), whereas there was no significant association between Hb and ferritin (r = 0.18; p = 0.38), folate (r = −0.19; p = 0.37), vitamin B_{12} (r = 0.24; p = 0.25), eGFR (r = 0.12; p = 0.58), or ln hs-CRP (r = −0.28; p = 0.15).

Whole-body and cardiac iron status in HF. In order to develop a biochemical assessment of whole-body iron turn-

Table 1 Clinical and Biochemical Characteristics According to Anemia

	Controls (n = 9)	HF − Anemia (n = 16)	HF + Anemia (n = 9)
Age, yrs	56 ± 15	51 ± 16	52 ± 9
Hemoglobin, g/l	140 ± 9	136 ± 4	103 ± 15†‡
LVEF, %	66 ± 9	22 ± 8*	26 ± 6‡
NT-proBNP, ng/l	53 (22–90)	2,551 (1,599–6,287)*	3,685 (1,174–6,764)‡
Mean arterial pressure, mm Hg	95 ± 15	79 ± 12*	71 ± 6‡
Cardiac output, l/min	6.4 ± 1.5	3.4 ± 0.8*	3.6 ± 0.9‡
PCWP, mm Hg	8 ± 3	24 ± 4*	21 ± 6‡
eGFR, ml/min/1.73 m^2	92 ± 24	70 ± 24	69 ± 21
Iron, μmol/l	18.4 ± 4.8	15.2 ± 6.8	7.1 ± 3.6‡†
Transferrin, g/l	2.47 (2.20–2.60)	2.79 (2.56–3.07)*	2.98 (2.62–3.19)‡
Transferrin saturation, %	30.3 ± 10.2	21.3 ± 8.8*	10.0 ± 6.6‡†
Soluble transferrin receptor, mg/l	2.9 (2.5–3.3)	4.4 (3.0–4.8)*	10 (4.5–13.9)‡†
High-sensitivity C-reactive protein, mg/l	1.1 (0.8–3.4)	5.8 (2.4–8.5)*	5.3 (1.8–9.4)

Values are mean ± SD or median (interquartile range). *p < 0.05 versus healthy control; †p < 0.05 versus nonanemic HF; ‡p < 0.05 versus healthy control.

eGFR = estimated glomerular filtration rate; HF = heart failure; LVEF = left ventricular ejection fraction; NT-proBNP = N-terminal pro–B-type natriuretic peptide; PCWP = pulmonary capillary wedge pressure.

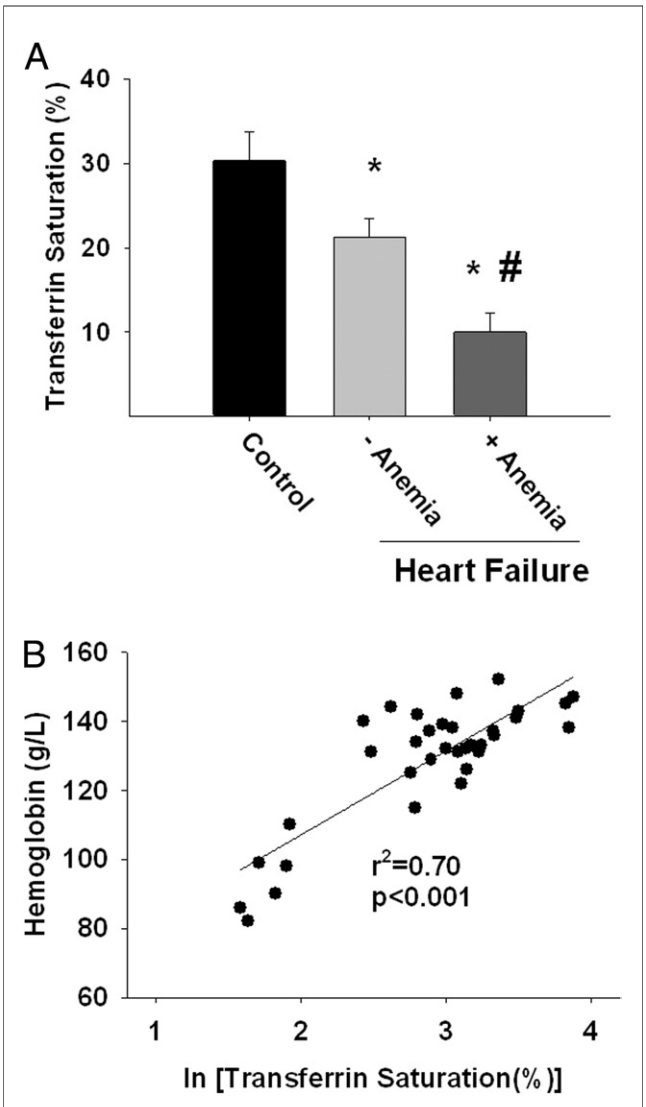

Figure 1 TFS in Healthy Subjects and HF Patients

(A) Bar graph showing transferrin saturation (TFS) in control patients and nonanemic and anemic heart failure (HF) patients. *$p < 0.05$ versus controls; #$p < 0.05$ versus nonanemic HF. Data are expressed as mean ± SEM. **(B)** Scatterplot with regression line demonstrating the relationship between ln-transformed transferrin saturation and hemoglobin.

over in the various patient groups, we calculated the arteriovenous difference in the TFS, given the greater sensitivity of the latter measure as an index of iron status. As shown in Figure 2, there was evidence of a net arteriovenous gradient in TFS in healthy controls, whereas little evidence existed in either of the HF cohorts, the differences being statistically significant.

In an attempt to directly investigate the uptake of iron by the myocardium, we determined the transcardiac concentration gradients for each relevant parameter. This analysis, however, showed only very small differences in concentrations for iron, transferrin, and TFS, likely due in part to the sensitivity of the respective assays together with the relatively small organ mass compared with body weight.

To extend our observations, we next determined the tissue content of iron in samples of nonfailing unused donor and failing human left ventricular myocardial samples, as determined by atomic adsorption spectroscopy. As shown in Figure 3, the tissue content of iron was significantly lower in the failing heart. Given the role of the transferrin receptor as the major uptake pathway for the transport of iron into the myocardium, we also examined the expression of Tfr1 in the nonfailing and failing human heart. As shown in Figure 3, we observed a 68% reduction ($p < 0.05$) in the level of Tfr1 mRNA expression.

Regulation of myocardial transferrin receptor expression. To investigate the potential basis for the observed downregulation of Tfr1 in the failing heart, we investigated the regulation of the receptor in isolated rat ventricular cardiomyocytes, using cell culture conditions of relevance to the HF paradigm. As shown in Figure 4, exposure to the neurohormones norepinephrine (10^{-7} mol/l) and aldosterone (10^{-7} mol/l) both resulted in a significant downregulation of mRNA for Tfr1. To establish the basis for the norepinephrine-induced decrease in Tfr1 expression, we compared the effects of 48-h exposure to the alpha- and beta-adrenoceptor agonists phenylephrine (10^{-6} mol/l) and isoprenaline (10^{-6} mol/l), respectively. As shown in Figure 4, the beta-agonist caused a 25% ($p < 0.05$) reduction in receptor expression, whereas the alpha-agonist exerted no apparent effect. The influence of B-type natriuretic peptide (10^{-7} mol/l) was also examined and found to be without effect on Tfr1 mRNA expression. In conjunction, we tested the effect of the neurohormones on Tfr1 mRNA levels in rat cardiac fibroblasts, and these were found not to alter gene expression.

Discussion

In the present study, we investigated the potential basis for the strong association between anemia and adverse progno-

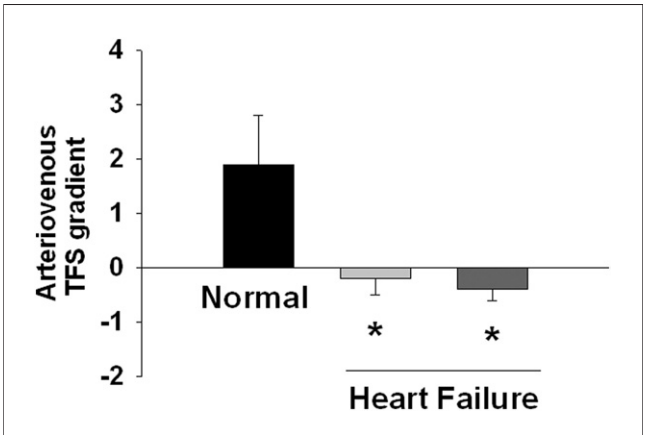

Figure 2 AV Differences in TFS

Bar graph depicting the arteriovenous (AV) difference in TFS in healthy subjects and nonanemic and anemic HF patients. *$p < 0.05$ versus controls. Data are expressed as mean ± SEM. Abbreviations as in Figure 1.

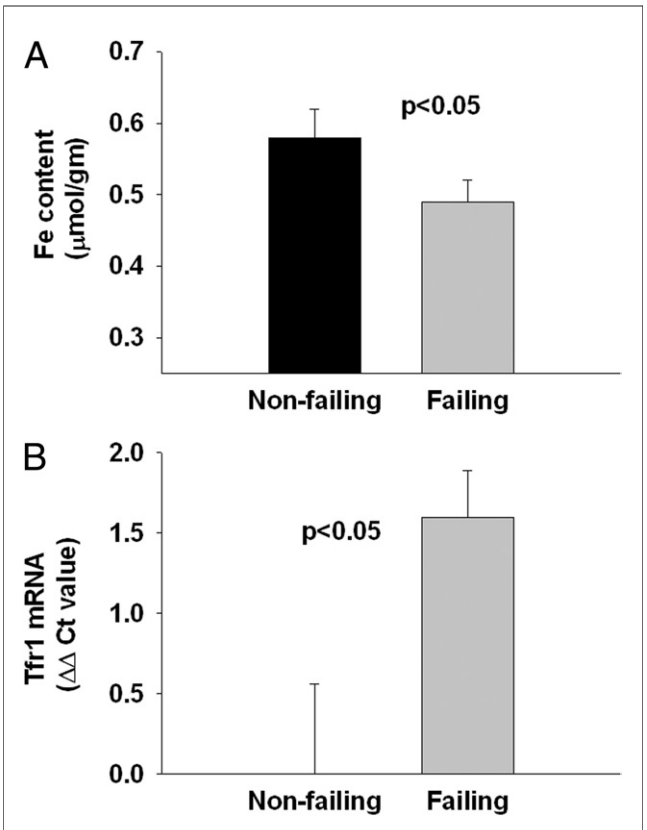

Figure 3 Myocardial Iron and Transferrin Receptor Expression

(A) Bar graphs comparing the iron content of failing and nonfailing human heart samples. **(B)** Bar graphs comparing the transferrin receptor mRNA abundance, expressed as ΔΔ Ct values (vs. control). Fold difference = $2^{-\Delta\Delta\ Ct}$. Data are expressed as mean ± SEM. Trfr1 = type 1 transferrin receptor.

sis in HF. Specifically, we hypothesized that iron deficiency per se may be the responsible mechanism rather than the effects of a low Hb itself. In our study, we observed that patients with advanced HF exhibited a lower Hb that was accompanied by biochemical indices of iron deficiency. When classified according to the presence or absence of anemia, HF patients displayed an even greater degree of iron deficiency, although nonanemic HF patients also had evidence of intermediate iron deficiency as measured by their TFS.

Previous studies of the prevalence of anemia in patients with HF report ranges between 15% and 70%, according to the setting of the study (1). Consistent with these studies, in the current study, 36% of our unselected, consecutive patients with advanced HF were anemic. As indicated in the previous text, the intense interest in the role of anemia in HF has arisen because of its association with adverse prognosis (3–7). It has also been reported from cross-sectional studies that anemia is associated with older age, renal impairment, and poorer hemodynamics (16), although we did not observe this in the present study.

Although the epidemiology of anemia in HF has been well characterized, the underlying mechanism remains poorly understood. Currently, 3 major mechanisms have been proposed for the anemia of HF, including impaired erythropoietin production or signaling, iron deficiency, or an anemia of chronic disease, perhaps due to the actions of inflammatory cytokines (1). Previous studies of erythropoietin in HF have yielded conflicting results, including the presence of high plasma levels (17). We did not measure the levels of erythropoietin in our cohort. The possibility that a relative deficiency of erythropoietin is the driving mechanism for anemia in HF has led to a number of trials, including ongoing large-scale trials. As recently reviewed (1), although therapy with erythropoiesis-stimulating agents resulted in a consistent rise in Hb in the larger studies (9,18), the impact on clinical measures has varied, and definitive outcome data are still awaited. These findings are of relevance to the hypothesis that the relationship between anemia and adverse outcome in HF is the result of the requirement for an increase in cardiac output to provide appropriate oxygen delivery. In our study, we did not find

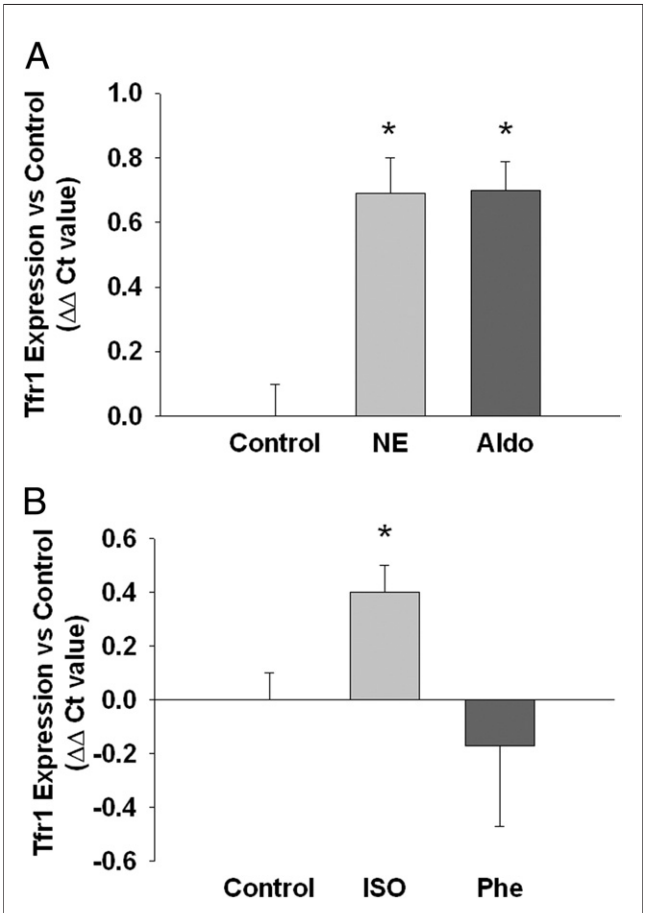

Figure 4 Regulation of Transferrin Receptors

(A) Bar graphs comparing transferrin receptor (Tfr1) mRNA expression in control and norepinephrine (NE) (10^{-7} mol/l) or aldosterone (Aldo) (10^{-7} mol/l) treated cardiomyocytes. **(B)** Bar graphs comparing transferrin receptor mRNA expression in control and isoprenaline (ISO) (10^{-6} mol/l) or phenylephrine (Phe) (10^{-6} mol/l) treated cardiomyocytes (n = 4). Data are expressed as ΔΔ Ct values (vs. control). Fold difference = $2^{-\Delta\Delta\ Ct}$. Data are expressed as mean ± SEM. *$p < 0.05$.

that cardiac output was significantly higher in anemic patients. This hypothesis is also potentially negated by the ability of the hemoglobin–oxygen dissociation curve to be modulated by other well-known intracellular means, including the levels of 2,3-diphosphoglycerate.

As indicated in the previous text, we demonstrated strong evidence for the presence of graded levels of iron deficiency in our HF patients, being most profound in the anemic group. As such, it is possible that anemia only becomes apparent in HF when total-body iron stores are sufficiently depleted, a concept that is supported by our demonstration of a strong relationship between TFS and Hb. Our population of patients with advanced HF was similar to the population with HF and anemia studied by Nanas et al. (10), who found that approximately three-quarters of these patients had an iron-deprived bone marrow despite normal ferritin. These authors concluded that anemia was due to absolute iron deficiency in the majority of cases (10). In our study, we also attempted to assess whole-body iron tissue uptake based upon the arteriovenous change in TFS. To the best of our knowledge, this parameter has not be measured before in either healthy subjects or controls. Previous investigators have used oral ^{59}Fe radiotracers to study gastrointestinal uptake; however, such studies (19) have tended to show varied uptake even in normal volunteers and with a limited relationship to hematinic parameters. In our study, the arteriovenous TFS gradient was significantly reduced in both nonanemic and anemic HF patients, suggesting a generalized reduction in iron uptake. However, others have argued that increased storage of iron in the reticuloendothelial system and decreased systemic iron availability could have resulted in iron depletion of the bone marrow despite normal total-body iron (11). From our data, it is not possible to tell whether patients had absolute iron deficiency (reduced iron stores) or relative iron deficiency (decreased systemic iron availability despite overall normal total/body iron). Other investigators have proposed that a chronic inflammatory state contributes to the anemia of HF; however, in the present study, we did not observe any differences in the levels of hs-CRP across any of the patient groups.

Based on our demonstration that iron stores are reduced in HF and that this likely reflects a generalized disorder, we next evaluated the possibility that a reduction in the content of iron in the myocardium may occur in HF. To the best of our knowledge, we showed for the first time that the iron content of the failing human heart is reduced compared with nonfailing hearts. In conjunction, we attempted to measure the transcardiac gradients for relevant parameters of iron metabolism; however, no consistent gradients were evident, likely due to the relatively small fractional uptake together with the limited sensitivity of the associated assays. Previously, it has been shown that animal models of iron deficiency develop progressive cardiac fibrosis (14) as well as other ultrastructural, macroscopic, and functional changes of the left ventricular myocardium (20–23), highlighting that cardiac iron uptake and intracellular iron handling may be critical for cardiac function. As such, it is possible that iron depletion in the failing human heart may contribute to disease progression. This concept could be consistent with the recent demonstration that iron supplementation in HF patients appears to be associated with improvements in clinical parameters (13) and, conversely, that iron deficiency appears to be an independent predictor of survival (8).

Iron plays a key intracellular role in many cell types, and in cardiac myocytes, this includes an important role within mitochondria and in myoglobin. In the circulation, iron is extensively carried by transferrin, the latter performing a role both as a transport protein and as a mechanism to maintain iron in a nonreactive state (24). In order for iron to be taken up by cells, transferrin binds to specific receptors, including Tfr1, which interacts with other intracellular proteins including beta2 microglobulin and an MHC class I-type protein encoded by the hemochromatosis-associated gene, *HFE*. In the present study, we observed reduced Tfr1 mRNA expression in the failing heart, providing in part a further explanation for the reduced levels of iron in the failing heart. This notion is further supported by previous studies demonstrating a close association between Tfr1 mRNA gene expression, Tfr1 protein abundance, and iron transport (25–27). To investigate the potential mechanism for this finding in the context of HF, we found that neurohormones that are increased in HF, aldosterone and norepinephrine, reduced the expression of Tfr1 in cardiomyocytes. Furthermore, the decrease in Tfr1 gene expression mediated by norepinephrine appeared to be predominantly mediated via the beta-receptor. In addition to the putative role of neurohormones as regulators of Tfr1, previous studies in nonmyocardial cells have shown that cytokines such as interferon-gamma can down-regulate Tfr1, whereas hypoxia inducible factor-1 can up-regulate Tfr1 (28).

Study limitations. Given the invasive nature of our study, we included a relatively limited number of healthy volunteers and HF subjects. Accordingly, although our study provided an opportunity to test our novel hypotheses, the limited sample size restricts our capacity to explore more detailed relationships between various factors that may contribute to alterations in iron homeostasis in vivo. One of our key objectives was to compare the uptake of iron in the normal and failing myocardium by evaluating the transcardiac concentration gradient for iron. No arteriovenous difference could be detected, presumably due to the combined limitation of assay sensitivity and the relatively small concentration gradient. To complement these studies, we also measured the myocardial iron concentration and Tfr1 gene expression, which revealed significantly lower levels of both in the failing heart. In this analysis, it is not possible to exclude an effect of replacement fibrosis; however, we demonstrated that unlike in cardiomyocytes, the expression of Tfr1 does not appear to alter in cardiac fibroblasts in the context of neurohormonal stimulation. We also acknowledge that the study is limited by the fact that we did not measure erythropoietin and reticulocyte counts,

which would have provided a more detailed characterization of the study population.

Conclusions

We provide evidence for both systemic and myocardial deficiency of iron in patients with advanced HF. Anemic patients displayed features of more pronounced iron deficiency, which may translate into a greater degree of iron depletion. Thus, further studies that incorporate the routine measurement of cardiac iron stores and the longitudinal effects of iron replenishment are warranted in HF patients.

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