Oral Iron Therapy for Heart Failure With Reduced Ejection Fraction

Design and Rationale for Oral Iron Repletion Effects on Oxygen Uptake in Heart Failure

Gregory D. Lewis, MD; Marc J. Semigran, MD; Michael M. Givertz, MD; Rajeev Malhotra, MD; Kevin J. Anstrom, PhD; Adrian F. Hernandez, MD, MHS; Monica R. Shah, MD; Eugene Braunwald, MD

Abstract—Iron deficiency is present in ≈50% of patients with heart failure and is an independent predictor of mortality. Despite growing recognition of the functional and prognostic significance of iron deficiency, randomized multicenter trials exploring the use of oral iron supplementation in heart failure, a therapy that is inexpensive, readily available, and safe, have not been performed. Moreover, patient characteristics that influence responsiveness to oral iron in patients with heart failure have not been defined. Although results of intravenous iron repletion trials have been promising, regularly treating patients with intravenous iron products is both expensive and poses logistical challenges for outpatients. Herein, we describe the rationale for the Oral Iron Repletion effects on Oxygen Uptake in Heart Failure (IRONOUT HF) trial. This National Institute of Health-sponsored trial will investigate oral iron polysaccharide compared with matching placebo with the primary end point of change in exercise capacity as measured by peak oxygen consumption at baseline and at 16 weeks.

Clinical Trial Registration—URL: http://www.clinicaltrials.gov. Unique identifier: NCT02188784. (Circ Heart Fail. 2016;9:e000345. DOI: 10.1161/CIRCHEARTFAILURE.115.000345.)

Key Words: clinical trial ■ exercise ■ heart failure ■ iron ■ oxygen consumption

Impaired exercise capacity is a cardinal manifestation of heart failure (HF) that is closely related to reduced quality of life and poor outcomes.1-3 Therapeutic options beyond neurohormonal blockade to improve functional capacity and symptoms in HF are currently limited and must be pursued. Iron deficiency is associated with reduced functional capacity and poorer quality of life in HF. Here, a rationale for oral iron repletion in patients with HF and iron deficiency is provided with an overview of the study design for the Iron Repletion effects on Oxygen Uptake in Heart Failure (IRONOUT HF) trial, which aims to study the effect of oral iron repletion on functional capacity in patients with HF and reduced ejection fraction (HFrEF).

Contributing Factors to Exercise Intolerance in Heart Failure

In patients with HF, multiple mechanisms contribute to exercise intolerance.4-8 Abnormal central hemodynamic responses and the degree of left ventricular systolic dysfunction do not adequately explain the early onset of anaerobic metabolism and impaired peak oxygen uptake ($V_{O2}$) observed in HF9-11 Prevalent anemia in HF compromises convective delivery of $O_2$ to exercising skeletal muscle. On delivery of $O_2$ to the periphery, diffusive $O_2$ conductance and utilization is limited by impaired skeletal muscle oxidative metabolism.12 Morphological and histochemical changes in skeletal muscle include a shift to type II fibers13 and a reduction in oxidative enzymes.13 As a result, patients with HF experience an early transition from oxidative to glycolytic metabolism and glycolytic end products, in turn, stimulate exaggerated ventilatory responses to exercise through intramuscle afferents sensitive to products of skeletal muscle work (ie, ergoreflex signaling).14 These findings have led to the concept of a central role of the peripheral as a dominant mechanism to explain breathlessness and exertional intolerance in HF.9,13 Strategies to target impaired peripheral utilization of oxygen during exercise, therefore, offer promise for improving functional capacity in HF.

Central Role of Fe in $O_2$ Delivery and Utilization

Iron plays a critical role in systemic $O_2$ delivery and utilization (Figure 1).16-23 The contribution of iron to erythropoiesis and the...
role of iron deficiency in decreasing \( \text{O}_2 \)-carrying capacity of the blood through reduced hemoglobin are widely recognized. A less well-appreciated consequence of iron deficiency is impairment in \( \text{O}_2 \) storage capacity in skeletal muscle through reduced myoglobin. In addition, iron is an obligate component of enzymes involved in cellular respiration, oxidative phosphorylation, vascular homeostasis, and the citric acid cycle (Table 1).

Animal studies have suggested that during iron repletion, improvements in hemoglobin levels and peak \( \text{VO}_2 \) evolve in parallel, whereas enhancements in endurance track with the increase in aerobic enzyme activity. Studies in animals and humans without HF have demonstrated that iron deficiency anemia reduces indices of work capacity by 10% to 50%. The correction of iron deficiency in both anemic and nonanemic patients without HF improves symptoms, quality of life, and exercise performance.

### Iron Homeostasis in Heart Failure

There are multiple factors that predispose patients with HF to iron deficiency (Figure 2). Reduced nutrient intake is common in HF, which may result in failure to meet the recommended 8 to 18 mg of daily oral intake of elemental iron. Impairment in iron homeostasis in HF is partially attributable to proinflammatory processes. Hepcidin, a hepatically derived peptide that is increased by proinflammatory cytokines, blocks intestinal absorption of iron and impairs iron delivery by diverting iron into the reticuloendothelial system. Gut edema may also impair the absorption of oral iron and contribute to iron deficiency in HF. Finally, frequent use of anticoagulants and antiplatelet agents predisposes patients with HF to blood loss and associated depletion in iron stores.

### Definition, Prevalence, and Significance of Iron Deficiency in HF

Historically, assessment of iron levels in patients with HF has been performed as part of the evaluation of anemia, which is often multifactorial in HF. However, recent studies have focused on the importance of iron deficiency independent from anemia in HF pathophysiology.

#### Definition

The gold standard for measurement of iron stores is a bone marrow biopsy. However, because of the invasiveness of this procedure, blood biomarkers, including ferritin and transferrin saturation, can be used instead to reflect iron bioavailability in patients. A ferritin value <30 ng/mL has historically been used as a cutoff for defining iron deficiency. However, the inflammatory processes in HF and their tendency to increase serum ferritin concentration cause iron deficiency in patients with HF to be widely under recognized using this definition. Therefore, for patients with HF, iron deficiency has been defined as either ferritin <100 ng/mL, indicating a deficiency in iron stores, or ferritin between 100 and 300 ng/mL with transferrin saturation <20%, suggesting a disruption in iron delivery.

Circulating levels of hepcidin and soluble transferrin receptor levels complement measurements of ferritin, iron, and total iron-binding capacity. Hepcidin inhibits iron absorption and bioavailability and is downregulated in primary iron deficiency, whereas soluble transferrin receptor levels are upregulated and

### Table 1. Fe-Containing Proteins That Are Altered in Heart Failure

<table>
<thead>
<tr>
<th>Name of Protein</th>
<th>Functional Site</th>
<th>Status in HF</th>
<th>Biological Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin</td>
<td>Red blood cell</td>
<td>↓</td>
<td>( O_2 ) transport</td>
</tr>
<tr>
<td>Myoglobin</td>
<td>Cytoplasm of muscle cells</td>
<td>↓</td>
<td>Facilitation of ( O_2 ) transport</td>
</tr>
<tr>
<td>Oxidative enzymes (ie, cytochrome oxidase)</td>
<td>Mitochondrial inner membrane</td>
<td>↓</td>
<td>Substrate oxidation ( \rightarrow ) NADH, FADH(_2)</td>
</tr>
<tr>
<td>Respiratory chain proteins</td>
<td>Mitochondrial inner membrane</td>
<td>↓</td>
<td>Electron transfer from ( O_2 ) ( \rightarrow ) NADH, FADH(_2)</td>
</tr>
<tr>
<td>Soluble guanylate cyclase</td>
<td>Vascular smooth muscle cells, cardiomyocytes</td>
<td>↓</td>
<td>Nitric oxide stimulation of cGMP synthesis</td>
</tr>
</tbody>
</table>

(cGMP indicates cyclic guanosine monophosphate; FADH\(_2\), reduced form of flavin adenine dinucleotide; HF, heart failure; and NADH, reduced form of nicotinamide adenine dinucleotide. Adapted with permission from Haas and Brownlie. Copyright © 2001, Wiley Brothers.)
released into the bloodstream by cells avid for iron during states of iron deficiency. Jankowska et al recently reported that hepcidin levels <14.5 ng/mL coupled with soluble transferrin receptor >1.6 mg/L are indicators of iron deficiency that independently predict poor prognosis in HF.

Prevalence
Approximately 70% of patients with HFrEF have reduced bone marrow iron stores, and 37% to 67% meet criteria to have iron deficiency. Although circulating ferritin levels can increase as an acute phase reactant, and thereby potentially mask iron deficiency in patients admitted with acute HF, a recent study found that 72% of hospitalized patients met diagnostic criteria for iron deficiency. Notably, 25% to 42% of patients with HF and iron deficiency are not anemic, which highlights the importance of considering iron deficiency even when hemoglobin levels are normal.

Functional and Prognostic Significance
Iron deficiency reduces indices of functional and endurance capacity in patients with HF which likely reflects its broad impact on oxygen transport and cellular oxidative capacity. Patients with HF and iron deficiency had a worse health-related quality of life (as assessed by Minnesota Living with Heart Failure questionnaire scores) than those without iron deficiency. In addition, iron deficiency was associated with poorer outcomes in patients with systolic HF, independent of other well-established prognostic factors, including anemia, New York Heart Association class, left ventricular ejection fraction, and N-terminal B-type natriuretic peptide levels.

Iron deficiency has also been associated with ultrastructural changes in cardiomyocytes, including mitochondrial swelling and abnormal sarcomere structure. Patients with HF have lower myocardial iron content and transferrin receptor concentrations than controls, and severe iron deficiency leads to LV systolic dysfunction. In a recent study of patients with nonischemic cardiomyopathy, reduced myocardial iron content (as defined by magnetic resonance imaging–based T2 star values in excess of 25) was associated with increased left ventricular end diastolic volume, decreased left ventricular ejection fraction, and poorer outcomes. Based on the multiple deleterious effects of iron deficiency on cardiac and peripheral performance, iron deficiency represents an attractive target to improve functional capacity in patients with HF.

Efficacy and Safety of Treatment of Iron Deficiency in HF
Clinical studies of intravenous iron repletion in HF are summarized in Table 2. Initial single-center trials focused on patients with HFrEF and iron-deficiency anemia, and consistently observed improvements in peak VO2, patient global assessment scores, and N-terminal B-type natriuretic peptide levels. Correction of iron deficiency has also been shown to have direct effects on myocardial contractility. Two meta-analyses reported significant improvement in echocardiography-derived left ventricular ejection fraction with iron therapy (mean improvement +5%). In another single-center study, an improvement in LV strain rate was reported after 3 months of intravenous iron therapy in 40 patients with iron deficiency and HFrEF. These findings reinforce the hypothesis that iron repletion may directly improve cardiac function in addition to promoting improved peripheral oxygen delivery and utilization.

The landmark randomized trial of iron repletion in patients with HFrEF and iron deficiency, Ferinject Assessment in Patients with Iron Deficiency-HF, demonstrated significant improvements in patient global assessment scores and decreases in New York Heart Association class with the administration of 200 mg intravenous ferric carboxymaltose weekly until repletion. Improvements in patient global assessment score and 6-minute walk distance were similar in patients with and without anemia. The Randomised, Double-blind
Table 2. Trials Evaluating Intravenous Iron Supplementation for Treatment of Heart Failure

<table>
<thead>
<tr>
<th>Drug</th>
<th>Authors/Journal</th>
<th>n Subjects Studied</th>
<th>Iron Deficiency Definition</th>
<th>Time</th>
<th>Primary End point</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous iron sucrose</td>
<td>Bolger et al²⁴/J Am Coll Cardiol 2006</td>
<td>16 NYHA 2–3 LVEF &lt;0.35</td>
<td>Ferritin &lt;400 ng/mL</td>
<td>12 wk</td>
<td>Δ 6MWD, ↓NYHA, ↓MLHF scores</td>
<td></td>
</tr>
<tr>
<td>Intravenous iron sucrose</td>
<td>Tobilli et al²/²/J Am Coll Cardiol 2007</td>
<td>40 NYHA 3–4 LVEF &lt;0.35</td>
<td>Ferritin &lt;100 ng/mL and Tsat &lt;20%</td>
<td>5 wk</td>
<td>Δ global assessment score</td>
<td>↑PGAS, ↑6MWD, ↓NT-BNP, ↓LVEF</td>
</tr>
<tr>
<td>Intravenous iron sucrose</td>
<td>Usmanov et al²/²/Nephrol 2008</td>
<td>32 NYHA 3–4 LVEF &lt;0.35</td>
<td>Hb &lt;11 g/dL Iron indices not specified</td>
<td>26 wk</td>
<td>Δ NYHA</td>
<td>↓NYHA, ↓LVEF</td>
</tr>
<tr>
<td>Intravenous iron sucrose</td>
<td>Okonko et al²/²/J Am Coll Cardiol 2008</td>
<td>35 NYHA 2–3 LVEF &lt;0.35</td>
<td>Ferritin &lt;100 ng/mL or 100–300 with Tsat &lt;20%</td>
<td>16 wk</td>
<td>Δ peak VO₂</td>
<td>↑PGAS, ↑NYHA, ↑peak VO₂ α, Tsat</td>
</tr>
<tr>
<td>Intravenous iron carboxymaltose</td>
<td>Anker et al²/²/N Engl J Med 2009</td>
<td>459 NYHA 2–3 LVEF &lt;0.4 Hb, 9.5–13.5</td>
<td>Ferritin &lt;100 ng/mL or 100–300 with Tsat &lt;20%</td>
<td>24 wk</td>
<td>Δ global assessment score</td>
<td>↑PGAS, ↑NYHA, ↑6MWD Similar benefit Hb &lt;12</td>
</tr>
<tr>
<td>Intravenous iron carboxymaltose</td>
<td>Ponikowski et al²/²/Eur Heart J 2014</td>
<td>304 NYHA 2–3 LVEF &lt;0.45 Hb &lt;15</td>
<td>Ferritin &lt;100 ng/mL or 100–300 with Tsat &lt;20%</td>
<td>52 wk</td>
<td>Δ 6MWD</td>
<td>↑6MWD, ↑PGAS, ↑NYHA Similar benefit Hb &lt; or &gt;12 ↓HF hospitalization</td>
</tr>
</tbody>
</table>

6MWD indicates 6-minute walk distance; 6MWT, 6-minute walk test; Hb, hemoglobin; HF, heart failure; LVEF, left ventricular ejection fraction; MLHF, Minnesota Living with Heart Failure; NT-BNP, N-terminal pro B-type natriuretic peptide; NYHA, New York Heart Association functional class; PGAS, patient global assessment score; Tsat, transferrin saturation; and VO₂, oxygen consumption.

Controlled Phase 4 Study to Compare the Efficacy and Safety of Intravenous Ferric Carboxymaltose With Placebo in Patients With Chronic Heart Failure and Iron Deficiency (CONFIRM-HF) trial, which assessed long-term therapy with intravenous ferric carboxymaltose in HFrEF, extended findings from Ferinject Assessment in Patients with Iron Deficiency-HF by demonstrating improvement in 6-minute walk distance and quality of life in patients with hemoglobin values ≤15 g/dL, while also demonstrating reduction in HF hospitalizations.³¹ The fact that iron repletion demonstrates similar efficacy in anemic and nonanemic patients clearly distinguishes this strategy from that of treatment of anemia alone with erythropoiesis-stimulating agents, which has been shown to not improve HF outcomes.⁴⁰

Oral Iron

With the exception of 1 truncated study that included 7 individuals randomized to oral iron,¹² trials exploring the use of oral iron supplementation in patients with HF and iron deficiency have not been performed. A recent retrospective observational study of 105 patients with HFrEF and iron deficiency showed that oral iron supplementation improved ferritin (median 39 to 75 ng/mL), Tsat (10% to 21%), iron (34 to 69 µg/dL), and hemoglobin (10.4 to 11.6 g/dL) values (all P<0.0001) during a median follow-up period of 164 days. In non-HF populations, intravenous and oral Fe repletion have resulted in similar sustained increases in circulating ferritin levels, transferrin saturations, and hemoglobin concentrations.⁵² A study in iron-deficient patients with congenital heart disease demonstrated functional improvements with oral iron supplementation after 3 months of iron fumarate (600 mg total daily dose).⁵² However, further investigation is clearly needed to determine the use of oral therapies in iron-deficient HF populations.

Rationale for IRONOUT

Despite growing recognition of the functional and prognostic significance of iron deficiency, current HF guidelines in the United States do not specify when, or whether, to assess for and treat iron deficiency. European guidelines have recently recommended evaluation of iron studies without specifically addressing whether to implement treatment strategies.

Although results of intravenous iron repletion trials have been promising, regularly treating patients with intravenous iron products is both expensive and poses logistical challenges for outpatients. In Ferinject Assessment in Patients with Iron Deficiency-HF, 8 to 12 weekly injections of intravenous iron (200 mg) were required to achieve iron repletion, followed by monthly injections during the maintenance phase. Intravenous iron infusions are expensive (≈$4000/injection for the least expensive preparation, iron sucrose 200 mg) and pose logistical challenges for outpatients based on the frequency of required visits and need for the infrastructure within HF clinics to administer infusions. Although a higher-dose intravenous iron preparation is now available (ferumoxytol [Feraheme], 510 mg elemental iron), this preparation has been associated with hypersensitivity reactions in 3.7% of patients, including anaphylaxis, cardiac arrest, and hypotension.⁵³ Randomized multicenter trials exploring the use of oral iron supplementation in HF, a therapy that is inexpensive, readily available, and safe, have not been performed. There is also a need to understand patient characteristics that influence responsiveness to oral iron. A recent retrospective study showed lack of relationship between dose of elemental iron and change in iron stores, suggesting host factors play an important role.⁵⁴ There may be subsets of patients with HFrEF (ie, lack of right HF and intestinal edema, low hepcidin levels) that benefit from oral iron supplementation even if the strategy is not effective in all.

Study Design

Oral Iron Repletion effects on Oxygen Uptake in Heart Failure (IRONOUT HF) is an National Institute of Health-sponsored multicenter, randomized, double-blinded, placebo-controlled trial of oral iron polysaccharide compared with matching placebo with the primary end point of change in peak VO₂ measured by cardiopulmonary exercise testing (CPET) at baseline.
Lewis et al Oral Iron for Heart Failure

and at 16 weeks (clinicaltrials.gov, NCT02188784, Figure 3). A total of 220 patients with HFrEF meeting the eligibility criteria (Table 3) are being enrolled and randomized in the study. Screening is being conducted in outpatients with chronic symptomatic HFrEF. Willing participants who are found to have iron deficiency and meet other entry criteria are being consented for the trial.

Randomization and Stratification

After providing written informed consent, research participants complete all baseline procedures, including clinical evaluation, blood samples, and CPET. Eligible participants are randomized with a 1:1 allocation ratio to either oral iron polysaccharide 150 mg twice daily or matching placebo. Randomization is stratified by anemia status (anemia is defined as hemoglobin <12 g/dL). A permuted block randomization method stratified by site is used to ensure relatively equal distribution of subjects to each arm within each clinical site.

Polysaccharide iron complex is being studied as the oral iron preparation of choice for this study because it is relatively nontoxic, thus permitting a higher therapeutic dosage (150–300 mg elemental iron daily) than other iron preparations. Polysaccharide iron complex capsules are a highly water-soluble complex of iron and a low-molecular weight polysaccharide. Iron polysaccharide is considered to be a dietary supplement, and although it has a human over-the-counter drug label, it is not an FDA approved drug. From previous studies using inclusion criteria based on the same iron study results, we estimated an average iron deficit of ≈1 g using the Ganzoni formula. Assuming 5% absorption of iron polysaccharide, a period of 10 weeks of 300 mg daily dosing is required to completely make up this deficit.

Participants are started on polysaccharide iron complex 150 mg or placebo administered twice daily in a double-blind fashion. Instructions are provided to take pills separately from meals and to avoid taking antacids, dairy products, tea, or coffee within 2 hours before or after this medication because they will decrease effectiveness. Drug administration with orange juice or other products rich in Vitamin C may enhance absorption and, therefore, is encouraged.

Primary End Point

Impaired exercise capacity is a cardinal manifestation of HF and it can be objectively and reproducibly measured by quantifying peak oxygen uptake (peak VO₂) through CPET. The multiple mechanisms by which iron repletion is expected to improve systemic O₂ delivery and utilization (Figure 1) are captured with assessment of peak VO₂, making this measure ideally suited to be the primary end point. Unlike changes in alternative trial end points, such as circulating biomarkers or echocardiographic parameters, there is significant intrinsic value to patients associated with improving exercise capacity.

For assessment of changes in exercise capacity in response to iron repletion, there are several advantages of CPET in comparison to 6-minute walk tests used in previous multicenter trials of intravenous iron repletion. First, CPET permits precise assessment of volitional effort based on whether the respiratory exchange ratio (VCO₂/VO₂) exceeds 1.0 during exercise; second, CPET provides insights into the organ system limiting gas exchange; third, CPET-derived peak VO₂ has been shown to outperform 6-minute walk distance in predicting HF outcomes; and finally, CPET permits measurement of an array of variables that reflect metabolic responses to low level, intermediate, and maximum exercise and thereby permits comprehensive assessment of the impact of a therapy that is expected to affect oxygen utilization via multiple mechanisms.

Secondary End Points and Exploratory Studies

Secondary end points of IRONOUT HF include assessments of the impact of oral Fe repletion on (1) submaximal exercise capacity, as measured by O₂ uptake kinetics on initiation of exercise; (2) ventilatory efficiency, as measured by minute ventilation relative to CO₂ production throughout exercise; (3) 6-minute walk distance; (4) plasma N-terminal pro B-type
Table 3. IRONOUT HF Inclusion and Exclusion Criteria

<table>
<thead>
<tr>
<th>Inclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≥ 18 y</td>
</tr>
<tr>
<td>Previous clinical diagnosis of heart failure with current NYHA class II–IV</td>
</tr>
<tr>
<td>symptoms, LVEF ≤ 40 within 2 y before consent, and ≥ 3 mo after a major change</td>
</tr>
<tr>
<td>in cardiac status (ie, CABG or CRT)</td>
</tr>
<tr>
<td>Serum ferritin between 15 and 100 ng/mL or serum ferritin between 100 and 299</td>
</tr>
<tr>
<td>ng/mL with transferrin saturation &lt; 20%</td>
</tr>
<tr>
<td>Hemoglobin 9.0–15.0 g/dL (males), 9.0–13.5 (females) at time of enrollment</td>
</tr>
<tr>
<td>Evidence-based medical therapy for HF (including β-blocker and ACE inhibitor/ARB</td>
</tr>
<tr>
<td>unless previously deemed intolerant and diuretics as necessary) with ≤ 100%</td>
</tr>
<tr>
<td>change in dose for 30 days before randomization. Changes in diuretic dose guided</td>
</tr>
<tr>
<td>by a patient-directed flexible dosing program are considered stable medical therapy</td>
</tr>
<tr>
<td>Willingness to provide informed consent</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exclusion criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of a neuromuscular, orthopedic, or other noncardiac condition that</td>
</tr>
<tr>
<td>prevents the patient from exercise testing on a cycle/treadmill ergometer and</td>
</tr>
<tr>
<td>inability to achieve an RER ≥ 1.0 on screening/baseline CPET</td>
</tr>
<tr>
<td>Severe renal dysfunction (eGFR &lt; 20 mL/min per 1.73 m²)</td>
</tr>
<tr>
<td>Severe liver disease (ALT or AST &gt; 3 × normal, alkaline phosphatase, or</td>
</tr>
<tr>
<td>bilirubin &gt; 2 × normal)</td>
</tr>
<tr>
<td>Gastrointestinal conditions known to impair Fe absorption (ie, inflammatory</td>
</tr>
<tr>
<td>bowel disease)</td>
</tr>
<tr>
<td>Known active infection as defined by current use of oral or intravenous antimicrobial agents</td>
</tr>
<tr>
<td>Documented active gastrointestinal bleeding</td>
</tr>
<tr>
<td>Active malignancy other than nonmelanoma skin cancers</td>
</tr>
<tr>
<td>Anemia with known cause other than iron deficiency or chronic disease</td>
</tr>
<tr>
<td>Fe overload disorders (ie, hemochromatosis or hemosiderosis)</td>
</tr>
<tr>
<td>History of erythropoietin, intravenous or oral Fe therapy, or blood transfusion</td>
</tr>
<tr>
<td>in previous 3 mo</td>
</tr>
<tr>
<td>Current ventricular assist device</td>
</tr>
<tr>
<td>Anticipated cardiac transplantation within the next 4 mo</td>
</tr>
<tr>
<td>Primary hypertrophic cardiomyopathy, infiltrative cardiomyopathy, acute</td>
</tr>
<tr>
<td>myocarditis, constrictive pericarditis, or tamponade</td>
</tr>
<tr>
<td>Previous adverse reaction to study drug or other oral Fe preparation</td>
</tr>
<tr>
<td>Known or anticipated pregnancy in the next 4 mo</td>
</tr>
</tbody>
</table>

ACE indicates angiotensin converting enzyme; ALT, alanine transaminase; ARB,     |
angiotensin receptor blockade; AST, aspartate transaminase; CABG, coronary      |
artery bypass surgery; CPET, cardiopulmonary exercise testing; CRT, cardiac      |
resynchronization therapy; eGFR, epidermal growth factor receptor; HF, heart     |
failure; IRONOUT HF, Oral Iron Repletion effects on Oxygen Uptake in Heart Failure; |
LVEF, left ventricular ejection fraction; and NYHA, New York Heart Association    |
functional class.

natriuretic peptide levels; and (5) health status: Kansas City Cardiomyopathy     |
Questionnaire. The following exploratory objectives, for which the trial is not     |
primarily powered, seek to determine if specific subgroups of patients derive        |
differential benefit from oral Fe polysaccharide: (1) patients with or without     |
anemia; (2) patients with or without venous congestion, based on jugular venous     |
pressure > 10 cm or lower extremity edema; and (3) patients with and without an RER ≥ 1.1 during maximum incremental exercise. Other exploratory objectives include whether iron repletion influences clinical outcomes: time to death and HF hospitalization or renal function (creatinine, cystatin C). The primary iron studies (iron, total iron-binding capacity, ferritin) will be measured at baseline and after 16 weeks of study medication to determine the extent to which oral iron leads to iron repletion in patients with HF.

Statistical Considerations

All primary analyses are based on the intention-to-treat principle. A general linear model with the change in peak VO2 measured at 16 weeks as the response variable and predictor variables including a treatment indicator and the baseline measure of peak VO2 are used in the primary analysis. Using a minimally important difference for peak VO2 of 1.0 mL/ (kg-min), and an estimate of 2.0 mL/(kg-min) for the SD for peak VO2 a sample size of 172 subjects (86 per group) provides 90% power to detect the minimally important difference with a 2-sided type I error of 0.05. Allowing for 20% missing data results in a sample size of 108 per group, or a total sample size of 220 subjects.

A secondary analysis of the peak VO2 outcome uses a repeated measures analysis. For this analysis, all measurements of peak VO2 (including baseline) are treated as response variables. The following covariates are included in the regression model: treatment group, time period, treatment group×time period interaction, baseline VO2, and baseline Fe level. An unstructured correlation matrix is assumed for the repeated measures within subjects.

General linear models and nonparametric approaches are used to analyze the continuous outcomes. For binary outcomes, χ2 tests and Fisher exact test are used for unadjusted comparisons. For adjusted comparisons, logistic regression analysis is used to compare oral iron versus placebo with the estimated odds ratio and associated 95% confidence interval.

Conclusions

Iron deficiency is present in ≈ 50% of patients with HFrEF and predicts poor prognosis independently of anemia.32 Despite these recent observations and trials suggesting benefits from intravenous iron repletion, current HF guidelines do not specify when or if to assess for and treat iron deficiency. The IRONOUT HF trial design has several strengths, including entry criteria that permit assessment of effects of oral iron repletion on patients with and without anemia, objective measurements of submaximum and maximum exercise capacity, novel secondary end points (ie, assessment of oxygen uptake kinetics) that directly reflect mechanisms of action of iron repletion, and careful patient phenotyping to aid in identification of subgroups that may benefit most from oral iron repletion.

Although the sample size and short duration of this trial precludes definitive assessment of patient outcomes, the results of IRONOUT HF could lead to guideline recommendations for use of oral iron to improve functional capacity in HF. Based on the low cost and widespread availability of this therapy, the trial is anticipated to affect the clinical practice of administering oral iron to patients with HF whether the
results are positive. If patients with HFrEF derive no benefit from oral iron repletion, it would mitigate polypharmacy that is nearly ubiquitous in patients with HF. However, if iron repletion is shown to improve exercise capacity beyond its effects on erythropoiesis, it will profoundly affect the treatment of HF.

Sources of Funding
This study was supported by grants from the National Heart, Lung, and Blood Institute (NHLBI; coordinating center: U10 HL110312, U10 HL110337, U10 HL110342, U10 HL110262, U10 HL110297, U10 HL110302, U10 HL110309, U10 HL110336, and U10 HL110338).

Disclosures
All authors acknowledge grant support from National Heart, Lung, and Blood Institute (NHLBI) during the conduct of this study. Dr Hernandez reports research funding from Amgen, AstraZeneca; Merck, Novartis and honorarium from Amgen; AstraZeneca; Luitpold; Merck, and Novartis. Dr Braunwald reports grant support and Blood Institute (NHLBI) during the conduct of this study.

References
Investigators. Beneficial effects of long-term intravenous iron therapy

Mareev V, McDonagh T, Parkhomenko A, Tavazzi L, Levesque V, Mori


Oral Iron Therapy for Heart Failure With Reduced Ejection Fraction: Design and Rationale for Oral Iron Repletion Effects on Oxygen Uptake in Heart Failure

Gregory D. Lewis, Marc J. Semigran, Michael M. Givertz, Rajeev Malhotra, Kevin J. Anstrom, Adrian F. Hernandez, Monica R. Shah and Eugene Braunwald

_Circ Heart Fail._ 2016;9:
doi: 10.1161/CIRCHEARTFAILURE.115.000345

_Circulation: Heart Failure_ is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2016 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-3289. Online ISSN: 1941-3297

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circheartfailure.ahajournals.org/content/9/5/e000345

Data Supplement (unedited) at:
http://circheartfailure.ahajournals.org/content/suppl/2016/05/02/CIRCHEARTFAILURE.115.000345.DC1
This is a License Agreement between Gregory Lewis ("You") and American Society for Nutrition ("American Society for Nutrition") provided by Copyright Clearance Center ("CCC"). The license consists of your order details, the terms and conditions provided by American Society for Nutrition, and the payment terms and conditions.

All payments must be made in full to CCC. For payment instructions, please see information listed at the bottom of this form.

<table>
<thead>
<tr>
<th>License Number</th>
<th>3833950290295</th>
</tr>
</thead>
<tbody>
<tr>
<td>License date</td>
<td>Mar 21, 2016</td>
</tr>
<tr>
<td>Volume number</td>
<td>131</td>
</tr>
<tr>
<td>Issue number</td>
<td>2</td>
</tr>
<tr>
<td>Licensed content publisher</td>
<td>American Society for Nutrition</td>
</tr>
<tr>
<td>Licensed content publication</td>
<td>The Journal of Nutrition</td>
</tr>
<tr>
<td>Licensed content title</td>
<td>Iron Deficiency and Reduced Work Capacity: A Critical Review of the Research to Determine a Causal Relationship</td>
</tr>
<tr>
<td>Licensed content author</td>
<td>Jere D. Haas, Thomas Brownlie IV</td>
</tr>
<tr>
<td>Licensed content date</td>
<td>Feb 1, 2001</td>
</tr>
<tr>
<td>Type of Use</td>
<td>Scholarly Journal</td>
</tr>
<tr>
<td>Requestor type</td>
<td>Academic Institution</td>
</tr>
<tr>
<td>Format</td>
<td>Electronic</td>
</tr>
<tr>
<td>Portion</td>
<td>Figures/table/illustration</td>
</tr>
<tr>
<td>Number of Figures/table/illustration</td>
<td>1</td>
</tr>
<tr>
<td>List of figures/table/illustration</td>
<td>Table 1</td>
</tr>
<tr>
<td>Will you be translating?</td>
<td>No</td>
</tr>
<tr>
<td>Territory of distribution</td>
<td>Worldwide</td>
</tr>
<tr>
<td>Order reference number</td>
<td>None</td>
</tr>
<tr>
<td>Title of new article</td>
<td>IRONOUT Methods Paper</td>
</tr>
<tr>
<td>Publication the new article is in</td>
<td>Circulation Heart Failure</td>
</tr>
<tr>
<td>Publisher of new article</td>
<td>Lippincott Williams &amp; Wilkins</td>
</tr>
<tr>
<td>Author of new article</td>
<td>Gregory Lewis</td>
</tr>
<tr>
<td>Expected publication date of new article</td>
<td>Apr 2016</td>
</tr>
<tr>
<td>Estimated size of new article (pages)</td>
<td>7</td>
</tr>
<tr>
<td>Total</td>
<td>0.00 USD</td>
</tr>
</tbody>
</table>
Terms and Conditions

Terms and Conditions for Rightslink Licenses

1) This license agreement ("Agreement") is between the Customer as identified by the user in Rightslink ("Customer") and The American Society for Nutrition, Inc, a nonprofit corporation with offices at 9650 Rockville Pike, Bethesda, Maryland 20814-3998 USA ("ASN"), regarding content published in one of the following ASN journals: The American Journal of Clinical Nutrition (AJCN) or The Journal of Nutrition (JN) or Advances in Nutrition. The license administrator for this Agreement is the Copyright Clearance Center (CCC).

2) Introduction
The publisher of this copyrighted material is ASN. By clicking "accept" in connection with completing this licensing transaction, you agree that the following terms and conditions apply to this transaction (along with the Billing and Payment terms and conditions established by CCC, at the time that you opened your Rightslink account and that are available at any time at http://myaccount.copyright.com.

3) ASN occasionally publishes material for which it does not hold copyright; in such instances, ASN identifies and properly cites the appropriate copyright holder. It is the Customer’s responsibility to verify that ASN holds copyright for which the Customer is requesting permission. If the material is credited to another source, then ASN does not have the authority to grant permission for its reuse and this license is to be considered invalid.

4) Limited License
ASN hereby grants to you a non-exclusive license to use this material. Licenses are for one-time use only with a maximum distribution equal to the number that you identified in the licensing process. Except when material is licensed for reuse in a scholarly journal, magazine, newsletter, newspaper, book, or textbook, in which case permission extends to reprints of the product produced for publisher restocking, any form of republication must be completed within one year from the date hereof (although copies prepared before then may be distributed thereafter). Reprints must not be new products nor marketed as such: reuse in new volumes or new editions must be licensed explicitly. Any form of republication must be completed within one year from the date hereof (although copies prepared before then may be distributed thereafter); and any electronic posting is limited to the time specified in this request, if any. ASN asks that commercial organizations limit distribution of photocopies to within the organization requesting permission. Requests to distribute content copyrighted by ASN outside of the requesting organization should be processed as reprints or ePrints. Use of more than 50% of an article copyrighted by ASN in a single new work must be reviewed separately by ASN staff.

5) Geographic Rights
Licenses may be exercised solely in the country or countries specified by the Customer on the Quick Price or Additional Data page in Rightslink. However, in cases in which ASN has exclusive republication licensees in specific countries, licenses to reproduce full-text material in these countries may be revoked by ASN. In such cases, the Customer would be referred to the license agent in the respective country or countries. For questions about whether this would apply to your order, please contact ASN at publications@nutrition.org.

6) Alterations/Modifications
Except for adaptations of tables and figures and for translations of content as described below, material may not be modified. Translations of figures, tables, and excerpts are
permitted as transactions within Rightslink. Translations of full-text materials must be approved by ASN before the Customer can proceed with the Rightslink transaction. Full-text translation requests processed through CCC will be considered invalid unless the Customer enters the ASN-issued approval code for the translation. A copy of all full-text translations must be sent to ASN.

7) Reservation of Rights
ASN reserves all rights not specifically granted in the combination of (i) the license details provided by you and accepted in the course of this licensing transaction, (ii) these terms and conditions, and (iii) CCC's Billing and Payment terms and conditions.

8) License Contingent on Payment
While you may exercise the rights licensed immediately upon issuance of the license at the end of the licensing process for the transaction, provided that you have disclosed complete and accurate details of your proposed use, no license is finally effective unless and until full payment is received from you (either by ASN or by CCC) as provided in CCC's Billing and Payment terms and conditions. If full payment is not received on a timely basis, then any license preliminarily granted shall be deemed automatically revoked and shall be void as if never granted. Further, in the event that you breach any of these terms and conditions or any of CCC's Billing and Payment terms and conditions, the license is automatically revoked and shall be void as if never granted. Use of materials as described in a revoked license, as well as any use of the materials beyond the scope of an unrevoked license, may constitute copyright infringement and publisher reserves the right to take any and all action to protect its copyright in the materials.

9) Copyright Notice: Disclaimer
You must give complete credit to the original source in connection with any reproduction or adaptation of the licensed material: 'Am J Clin Nutr (year;volume:page range), American Society for Nutrition,' or 'J Nutr (year;volume:page range), American Society for Nutrition;' or 'Adv Nutr (year;volume:page range), American Society for Nutrition.' For translations: Figures or tables cannot be used in advertisements.

Full-text translations of articles published in The Journal of Nutrition must include the following disclaimer: Translated from the original into LANGUAGE by TRANSLATOR'S NAME. The translator assumes responsibility for the accuracy of the translation. The American Society for Nutrition is not responsible for translation errors. Readers are encouraged to access the original publication at http://jn.nutrition.org. For photocopies and in-house reprints:
The article will be reprinted in its entirety without change.
No material will be attached to the reprints, and the reprint will be used for educational purposes only, not to promote, sell, or in any way endorse a product.
The citation appearing on the first page of the article must appear in the reprints.
As a courtesy, please notify the author of your intended reuse of his or her content.
All reprints must have reprinted on each: The American Society for Nutrition, Inc., does not endorse any commercial enterprise.

10) Warranties
ASN makes no representations or warranties with respect to the licensed material.

11) Indemnity
You hereby indemnify and agree to hold harmless ASN and CCC, and their respective officers, directors, employees, and agents, from and against any and all claims arising out of your use of the licensed material other than as specifically authorized pursuant to this
license.

12) No Transfer of License
This license is personal to you, but may be assigned or transferred by you to a business
associate (or to your employer) if you give prompt written notice of the assignment or
transfer to ASN. No such assignment or transfer shall relieve you of the obligation to pay the
designated license fee on a timely basis (although payment by the identified assignee can
fulfill your obligation).

13) No Amendment Except in Writing
This license may not be amended except in a writing signed by both parties (or, in the case
of ASN, by CCC on ASN’s behalf).

14) Objection to Contrary Terms
ASN hereby objects to any terms contained in any purchase order, acknowledgment, check
endorsement or other writing prepared by you, which terms are inconsistent with these terms
and conditions or CCC's Billing and Payment terms and conditions. These terms and
conditions, together with CCC's Billing and Payment terms and conditions (which are
incorporated herein), comprise the entire agreement between you and ASN (and CCC)
concerning this licensing transaction. In the event of any conflict between your obligations
established by these terms and conditions and those established by CCC's Billing and
Payment terms and conditions, these terms and conditions shall control.

15) Jurisdiction
This license Agreement contains the entire understanding of the parties with respect to the
licensed content and can be modified only by a signed, written agreement. This Agreement
shall be construed in accordance with the laws of the State of Maryland and the US
copyright laws. If any term of this Agreement shall be found invalid by any court of
competent jurisdiction, such provision shall be enforced to the fullest extent that it is valid
and enforceable under applicable law, and all other provisions of this Agreement shall
remain in full force and effect. For state and local governments, the Terms and Conditions
do not apply when in conflict with existing statues.

16) Other
There are additional terms and conditions, established by Copyright Clearance Center, Inc.
("CCC") as the administrator of this licensing service that relate to billing and payment for
licenses provided through this service. Those terms and conditions apply to each transaction
as if they were restated here. As a user of this service, you agreed to those terms and
conditions at the time that you established your account, and you may see them again at any

Terms and Conditions for Content Services

Subject to these terms of use, any terms set forth on the particular order, and payment of the
applicable fee, you may make the following uses of the ordered materials:

Content Rental: You may access and view a single electronic copy of the materials ordered
for the time period designated at the time the order is placed. Access to the materials will be
provided through a dedicated content viewer or other portal, and access will be discontinued
upon expiration of the designated time period. An order for Content Rental does not include
any rights to print, download, save, create additional copies, to distribute or to reuse in any
way the full text or parts of the materials.
**Content Purchase:** You may access and download a single electronic copy of the materials ordered. Copies will be provided by email or by such other means as publisher may make available from time to time. An order for Content Purchase does not include any rights to create additional copies or to distribute copies of the materials.

The materials may be accessed and used only by the person who placed the Order or the person on whose behalf the order was placed and only in accordance with the terms included in the particular order.

Special Terms: `<%=specialTerms%>`

v 2.6

**Questions?** [customercare@copyright.com](mailto:customercare@copyright.com) or +1-855-239-3415 (toll free in the US) or +1-978-646-2777.