King's College Hospital NHS Foundation Trust

KING'S College LONDON

University of London

Ferric-HF II

Ferric Iron in Heart Failure

CLINICAL STUDY REPORT

Trial (Full Title):	Ferric Iron in Heart Failure (FERRIC-HF) II Trial: A Phase IV Double-Blind, Placebo-Controlled, Parallel MechanisticStudy to Assess the Mechanism(s) of Exercise Benefit with Intravenous Iron in Chronic Heart Failure.
Trial (Short Title):	FERRIC-HF II
EudraCT Number:	2012-005592-13
REC Number:	13/SC/0089
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GCP Statement:	This study was performed in compliance with ICH Good Clinical Practice (GCP) including the archiving of essential document.
Date of Report:	02/04/2018

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1. BACKGROUND AND RATIONALE TO FERRIC-HF II

Iron deficiency is common in chronic heart failure (CHF) and associated with greater symptoms, exercise intolerance and mortality even in the absence of anaemia. Intravenous (IV) iron repletion improves exercise performance in CHF but the mechanisms of benefit remain obscure. Besides haemoglobin (Hb), iron is an obligate component of pivotal metalloproteins involved in cellular energy production such as all enzymes of the electron transport chain (oxidative phosphorylation) and most mediators of the Krebs cycle and glycolytic pathways (glucose metabolism).

Because IV iron improves exercise capacity even in the absence of a rise in Hb in anaemic and non-anaemic patients, augmented mitochondrial oxidative capacity may be the dominant mechanism of benefit in CHF. To date, this hypothesis remains untested. ^{31}Phosphorous magnetic resonance spectroscopy (^{31}P-MRS) is a non-invasive tool that quantifies muscle oxidative capacity by measuring phosphocreatinine (PCr) and adenosine diphosphate (ADP) recovery half times ($t_{1/2}$) after exercise.

We conducted the FERRIC-HF II trial to test the hypothesis that IV Iron repletion improves skeletal muscle oxidative capacity as measured by PCr $t_{1/2}$ using ^{31}P-MRS in anaemic and non-anaemic CHF patients.

2.0 STUDY DESIGN

This was a prospective, single-centre, randomized, placebo-controlled, double-blinded, parallel-group, mechanistic study (see **Appendix 1 & 2** for study design, treatment schema and schedule of assessments).

Total planned enrolment was 40 subjects at a single centre (King's College Hospital) in Europe.

3.0 STUDY OBJECTIVES

3.1 Primary Objective

To evaluate the effect of IV iron repletion on skeletal muscle oxidative capacity as quantified by PCr $t_{1/2}$ using ^{31}P-MRS.

3.2 Secondary Objectives

To evaluate the effect of IV iron repletion on the following variables:

- Skeletal muscle oxidative capacity as assessed on ^{31}P-MRS by ADP $t_{1/2}$, levels of phosphorous compounds (ADP, PCr, and inorganic phosphate [Pi]), and pH.
- Symptoms as reflected by NYHA class and visual analogue fatigue scale
- Quantify of life as reflected by the Kansas City Cardiomyopathy questionnaire(KCCQ)
- Exercise capacity assessed by 6 minute walk distance and Pre- and Post-test Borg levels.
- Exercise capacity assessed by cardiopulmonary exercise parameters such as peak oxygen consumption [VO_2]
- Blood markers (iron status, haematological indices, N-terminal brain natiuretic peptide [NT-BNP], and cytokines)
- Cardiac function on echocardiography
- Skeletal muscle mitochondrial oxygen consumption per mg of muscle tissue measured using respirometry on muscle biopsy specimens.
- Skeletal muscle fibre type on immunohistochemistry, aerobic enzyme levels and iron status
- Erythroid precursor and progenitor [BFU-E] numbers
- Vital parameters
- Kidney and liver function tests
- Adverse events

4 SUBJECT ELIGIBILITY

4.1 Inclusion Criteria

- ≥30 years of age and have signed written informed consent
- Stable symptomatic CHF; New York Heart Association (NYHA) III/IV and left ventricular ejection fraction (LVEF) ≤45%, or if NYHA II then LVEF must be ≤40% as assessed within last 6 months using echocardiographic or magnetic resonance imaging techniques.
- On optimal conventional therapy for at least 4 weeks prior to recruitment and without dose changes for at least 2 weeks.
- Screening Hb concentration < 12 g/dl in females and < 13 g/dL in males (anaemic group, 50% of study population) or ≥12 g/dL in females and ≥ 13 g/dL in males (non-anaemic group, 50% of study population).
- Ferritin <100 μg/l or 100-300 μg/l with transferrin saturation (TSAT) <20%.
- Folate and Vitamin B_{12} levels ≥ lower limit of normal (according to local lab reference range).

- Resting blood pressure ≤160/100 mmHg.
- Negative pregnancy test in women of child-bearing age

4.2 Exclusion Criteria

- History of acquired iron overload, known haemochromatosis or first relatives with haemochromatosis, and allergic disorders (asthma, eczema, and anaphylactic reactions).
- Known hypersensitivity to parental iron preparations or any of their excipients.
- Known active infection, bleeding, malignancy, haemolytic anaemia, and rheumatoid arthritis.
- History of chronic liver disease with alanine transaminase (ALT) or aspartate transaminase (AST) >3 times the upper limit of the normal range, severe chronic lung disease with FEV_1 < 50% predicted, myelodysplastic disorder, and known HIV/AIDS disease.
- Coagulopathy or anticoagulation with warfarin or warfarin substitutes for a metallic valve or an LV thrombus diagnosed in the last 6 months.
- Contraindications to magnetic resonance imaging (pacemaker, cardiac resynchronization therapy device, internal cardiac defibrillator, metal prostheses, excessive claustrophobia)
- Recipient of immunosuppressive therapy or renal dialysis.
- Anticipated need for erythropoietin or a blood transfusion during the study.
- Unstable angina as judged by the investigator, severe uncorrected non-functional valvular disease or left ventricular outflow obstruction, obstructive cardiomyopathy, uncontrolled fast atrial fibrillation or flutter (>110 bpm), uncontrolled symptomatic brady- or tachyarrhythmias.
- Musculoskeletal limitation that, in the investigators judgement, would impair exercise testing.
- Pregnant or breast-feeding
- Inability to comprehend study protocol
- Parallel participation in another clinical trial

5.0 TREATMENTS

5.1 Treatment Arms

Subjects enrolled in the study were randomised to a total repletion dose of IV iron isomaltoside 1000 (Monofer®; IIM) or normal saline placebo.

5.2 Randomisation

Eligible patients were randomised 1:1 to IIM or saline placebo within 2 strata (anaemic and non-anaemic). This was performed by a clinical trials pharmacist using an automated, web-based, computer-generated list (Sealed Envelope Ltd, London, UK).

5.3 Blinding

Allocated treatment was dispensed to an unblinded nurse who achieved patient blinding by using opaque IV bags and giving sets for the infusions, and shielding the infusion arm from the patient. The unblinded nurses were not involved in the assessment of endpoints.

6.0 STATISTICS

Statistical analyses were as prespecified in version 2 of the analysis plan and followed the intention-to-treat principle. Sample size estimates were hampered by a paucity of quadriceps ^{31}P MRS data in patients with chronic HF. Initial calculations based on a trial using calf ^{31}P MRS, suggested that 40 patients in total would be needed to detect a 30 sec difference in PCr $t_{1/2}$ (α = 0.05, β = 90%, standard deviation = 24 sec) and cover for drop-outs and missing data. Review of the accruing blinded data suggested that the variance and standard deviation of PCr $t_{1/2}$ were likely to be smaller but that 40 patients in total was still sufficient to detect a more conservative difference of 6 sec with a standard deviation of 5 sec (α = 0.05, β = 90%).

The primary analysis was a comparison of PCr $t_{1/2}$ at 2 weeks using an analysis of covariance (ANCOVA) model with baseline PCr $t_{1/2}$ as covariate. All other continuous endpoints were similarly analysed. Missing data were imputed using the last observation carry-forward method for patients who had a last observation recorded post treatment. For those who did not, the mean value for their treatment group was used for imputations. Sensitivity analyses without imputation and with a *post-hoc* Markov chain-Monte Carlo imputation with 10 iterations were performed. Categorical variables were evaluated using a Pearson's χ^2 test. Baseline and week-2 data are described using appropriate summary measures. The estimates and two-sided 95% confidence intervals (95% CI) for the difference between least-squares means of the two treatment arms are given. All statistical tests were two-sided and we judged a P-value < 0.05 significant. All analyses were carried out using Stata 9.2 (Statacorp, Texas) and Statview 4.5 for Windows (Abacus Concepts, California).

7.0 GOOD CLINICAL PRACTICE

7.1 Regulatory Approvals

- South-Central Berkshire NHS National Research Ethics Service gave ethical approval (13/SC/0089) on 10/05/2013.
- Medicines and Health Regulatory Authority gave regulatory approval (14523/0252/001-0001) on 27/02/2014.
- King's College Hospital NHS foundation trust local R&D approved the study (KCH14-138) on 22/08/2014
- The sponsor (KHP-CTO) gave the green light for trial commencement on 24/10/2014 with the first patient screened on 28/10/2014.

7.2 Protocol Amendments

The protocol was substantially amended from version 6 to 9 prior to first subject enrollment on 06/01/2014. Versions 7 and 8 were internal iterations. Key changes with this revision included:

- Change of sponsor and legal representative from Imperial College London to King's College London and King's College Hospital NHS Foundation Trust. This reflected movement of the chief investigator from Imperial to King's College London.
- Change of method for measuring the primary endpoint (muscle oxidative capacity) from *ex-vivo* respirometry to *in-vivo* ^{31}P-MRS as this is a more reproducible and physiologically relevant method.
- Addition of cardiopulmonary exercise testing as a secondary endpoint
- Clarification that erythroid precursor cell (BFU-E) quantification would be part of the haematological assessments.
- Change of randomisation method from envelopes to a more stringent automated process.

The protocol was further substantially amended from version 9 to 10 after subject enrolment had commenced on 20/07/2015 and included the following alterations:

- Clarification that only folate and vitamin B12 levels below the lower limit of normal were an exclusion criteria as levels above the upper limit are not physiological adverse.
- Clarification that chronic lung disease would only be a contraindication if patients had objective measures of severe lung disease (FEV1<50% predicted).
- Clarification that severe heart valve regurgitation was only a contraindication if it was secondary to structural valve disease and not due to functional valve disease.

- Clarification that a high AST is only a contraindication if patients have chronic liver disease unrelated to heart failure.

A non-protocol related minor amendment was made on 22/04/2016 to include Wexham Park Hospital and Lewisham Hospital as participant identification centres. These sites were never used as all subjects were recruitable from King's College Hospital.

A final minor protocol amendment was made from version 10 to 11 on 31/10/2016 to widen the visit windows slightly to accommodate the logistics of the trial. The trial was also extended from 01/12/16 to 31/03/17.

All amendments were approved by the relevant regulatory authorities prior to implementation.

7.3 Statistical Analysis Plan Amendment

The statistical analysis plan was amended from version 1 to 2 on 06/10/2014 to reflect the fact that an unpaired t-test was an outdated and increasingly inappropriate means of comparing change in variables over time in parallel-group prospective trials. An ANCOVA comparison of the end of study values between groups after adjustment for their baseline values was deemed the better and more precise statistic for assessing change over time data, partly because it caters for regression to the mean which can confound analyses.

7.4 Data Management and Quality Assurance

Subject data were recorded by authorized site personnel on electronic CRF's that were set-up and maintained by the sponsors (KHP-CTO). Inputted data were scrutinized for discrepancies by KHP-CTO monitors. All discrepancies were subsequently settled prior to database lock.

8.0 RESULTS

8.1 Trial Timelines and Patient Disposition

The trial met its randomisation target of 40 patients. The first patient was screened on 28/10/2014 and the first randomised treatment was dispensed on 22/01/2015. Trial disposition is depicted in **Appendix 3**. The last randomised patient's week 2 visit, defined by the protocol as the end of the trial, was on 20/12/2016. Subsequent batch analysis of non-routine bloods and muscle biopsies, and complete database entry and verification then ensured.

8.2 Protocol Deviations

Appendix 4 provides a breakdown of patient-specific protocol deviations which were all corrected by amending the protocol or the relevant defective document. Additional deviations included 3 members of staff been incorrectly crossed out of the delegation log after they had left the trust, and erroneous skipping of screening number 47 and randomisation number 9.

8.3 Baseline Characteristics of Study Population

Appendix 5 provides the baseline characteristics. In the total population, mean age was 66±12 years and 30% were female. Baseline characteristics were generally similar between the two treatment groups. All patients received their allocated therapy at a single sitting except for 1 subject who received placebo over 2 sittings. In those randomized to IIM, the mean iron repletion dose was 929±320 mg (11±4 mg/kg). No subject was lost to follow-up and only 1 patient did not attend an end-of-study visit due to hospitalization. Overall, 4.2% of the analysed data was imputed. Sensitivity analyses yielded consistent results.

8.4 Primary Endpoint

During dynamic ^{31}P MRS the treatment arms exercised against similar weights (IIM: 5.7±0.7 kg, placebo: 5.5±0.9 kg, P=0.57) and achieved similar degrees of exertional PCr depletion at baseline (IIM: 36±11%, placebo: 34±13%, P=0.52) and at 2 weeks (IIM: 38±13%, placebo: 37±9%, P=0.71). At baseline, post-exercise PCr $t_{1/2}$ was similar in the randomized groups (**Appendix 5**). After treatment, PCr $t_{1/2}$ improved (shortened) by 17% (-4±10 sec) in the IIM group and worsened by 7% (3±7 sec) in the placebo arm (**Appendix 6**). The primary endpoint, PCr $t_{1/2}$ at 2 weeks, was significantly shorter in patients randomized to IIM with an adjusted between-group difference of –6.8 sec (95% CI -11.5 to -2.1, P=0.006; **Appendix 6 and 7**). This remained significant even after *post-hoc* adjustment for both baseline PCr $t_{1/2}$ and Hb (-6.8 sec, 95% CI -11.6 to -2.0, P=0.007) or other variables that appeared unbalanced such as age (-8.0 sec, 95% CI -12.8 to -3.2, P=0.002), and NTpro-BNP (-7.2 sec, 95% CI-10.5 to -1.0, P=0.007). No anaemia status by treatment group interaction existed (P=0.44). Baseline PCr $t_{1/2}$ was missing in 2 IIM patients and 1 placebo patient. Week 2 PCr $t_{1/2}$ was missing in 2 IIM patients. These were missing due to equipment malfunction or patient hospitalisation.

8.5 Secondary Efficacy Endpoints

Secondary efficacy endpoint results are shown in **Appendix 6**. Treatment with IIM significantly improved post-exercise skeletal muscle ADP $t_{1/2}$, but had no effect on resting or end-exercise metabolite (PCr, ADP) or pH levels on ^{31}P MRS. As expected, changes in ADP $t_{1/2}$ correlated with changes in PCr $t_{1/2}$ (r=0.65, P<0.001). Therapy with IIM also improved symptoms as reflected by reductions in NYHA class and the post-exercise Borg dyspnea score, but had no effect on the visual analogue fatigue scale (-0.59, 95% CI -1.45 to 0.28, P=0.18) or quality of life as reflected by the KCCQ.

Symptomatic improvements with also not accompanied by changes in cardiac structure and function (as reflected by LVEF), NT-proBNP, and exercise capacity as assessed by the 6-min walk distance, peak VO_2, or the ratio of minute ventilation to CO_2 production (VE/VCO_2 slope: -0.2, 95% CI -3.3 to 2.9, P=0.88) despite both groups attaining similar peak respiratory exchange ratios at baseline and at week 2 (IIM: 1.10±0.10, placebo: 1.05±0.09). Changes in 6-min walk distance related to changes in PCr $t_{1/2}$ (r= -0.33, P=0.04), and peak VO_2 related to PCr $t_{1/2}$ in the IIM (r = -0.57, P=0.007) but not placebo (r = - 0.30, P=0.21) group at 2 weeks.

Blood iron status as reflected by Ferritin and TSAT increased with IIM, but Hb remained unchanged. Reticulocyte counts and oxidative stress markers were not measured due to technical issues. Changes in ferritin (log transformed; r = - 0.37, P=0.02) but not Hb (r=0.17, P=0.30) correlated with changes in PCr $t_{1/2}$. Levels of the cytokines tumor necrosis factor (TNF; -0.01pg/ml, 95% CI -0.81 to 0.79, P=0.98), soluble TNF receptor 1 (-31 pg/ml, 95% CI -234 to 172, P=0.76), soluble TNF receptor 2 (34 pg/ml, 95% CI -342 to 411, P=0.86), interleukin-6 (4 pg/ml, 95% CI -5 to 13, P=0.40), interleukin-1 (1 pg/ml, 95% CI -2 to 4, P=0.43), and gamma-interferon (0.13 pg/ml, 95% CI -0.12 to 0.39, P=0.29) were unaltered by IIM.

Treatment with IIM did not alter skeletal muscle succinate-driven maximal oxygen consumption (0.01pmol O_2/sec/mg, 95% CI -0.11 to 0.14, P=0.85), %oxidative slow fibres on histochemisty (-2.16%, 95% CI - 9.29 to 4.96, P=0.54), and levels (all P>0.05) of aerobic enzymes (Complex I, II, III, IV & V), other muscle proteins (Myoglobin, Transferrin receptor, Ferritin, PGC-1alpha, Aconitase, HIF-1alpha), and glycophorin positive erythroid precursor numbers. Repeat muscle biopsies were not done in 7 patients (5 IIM patients and 2 placebo patients) due to patient hospitalisation (n=1), lack of attendance (n=1), or refusal (n=5). In all but 3 patients, no erythroid progenitor colonies grew in cell culture.

8.6 Safety Endpoints

The incidence of adverse events was comparable between the treatment arms with 3 (14%) and 1 (5%) events in the IIM and placebo groups, respectively (P=0.34). In the IIM arm, 1 patient had arthralgia during the infusion, 1 patient noted a mild rash at the venepuncture site 1 day post-infusion, and 1 patient had a serious adverse event (see below) that was judged unrelated to study drug. No anaphylactic reactions occurred. In the placebo arm, 1 patient reported coryzal symptoms 3 days post-infusion.

Infusions of IIM had no impact on haemodynamics. Systolic blood pressure remained unchanged from pre-infusion (122±17 mmHg) to 15 (123±17 mmHg), 30 (122±17 mmHg), 45 (119±19 mmHg), and 60 (121±15 mmHg) minutes post-infusion (all P>0.05). Diastolic blood pressure and heart rate were similarly stable. At 2 weeks, no differences between the treatment arms were seen for blood pressure, heart rate, and serum creatinine, AST or CRP levels, but IIM was associated with significantly reduced respiratory rates (**Appendix 6**).

8.7 Subgroup Efficacy Analysis

Baseline characteristics for the anaemic and non-anaemic subgroups stratified by treatment allocation are shown in **Appendix 5**. Mean iron repletion dose was 1155±221 mg (13±2 mg/kg) in anaemic patients and 680±204 mg (9±3 mg/kg) in non-anaemic patients. Baseline PCr $t_{1/2}$ was similar in the randomized groups in both anaemic and non-anaemic subpopulations. Infusion of IIM significantly improved (shortened) PCr $t_{1/2}$ at 2 weeks in anaemic patients. A smaller improvement in non-anaemic subjects did not reach statistical significance (**Appendix 8**).

8.8 Serious Adverse Events

A serious adverse event occurred in only 1 patient (randomisation number 31) who was hospitalised with a heart attack and lung congestion 1 week after receiving his assigned therapy. He had a background history of prior multiple heart attacks and went on to have coronary artery bypass grafting surgery during this reported hospitalisation. A form reporting this serious adverse event was submitted to the sponsor, KHP-CTO, within 24 hours of occurrence. In the opinion of the investigators, the event was thought highly unlikely to be related to randomised therapy. The patient recovered with no persistent complication.

9.0 CONCLUSION

The FERRIC-HF II trial was successfully concluded. Patient enrolment and follow-up was 100% with only 1 serious adverse event occurring. The trial found that, in iron-deficient patients with chronic HF and an LVEF ≤45%, a single total dose infusion of IIM safely repleted iron stores and was associated with faster skeletal muscle post-exercise PCr recovery kinetics at 2 weeks, implying better mitochondrial function. Enhancements in skeletal muscle energetics occurred despite no change in Hb, but were paralleled by improvements in symptoms as reflected by reductions in NYHA class and the post-exercise Borg dyspnea score. Augmented skeletal muscle energetics might therefore be an important mechanism via which iron repletion improves functional capacity despite eliciting minimal Hb changes. Ethical approval for additional post-hoc skeletal muscle tissue analyses will be sought as the molecular basis of the energetic enhancements seen in FERRIC-HF II were not revealed by the endpoints chosen in the protocol. Post-hoc alternative means of analysing the molecular data might also shed light.

10.0 DECLARATION & SIGNATURE

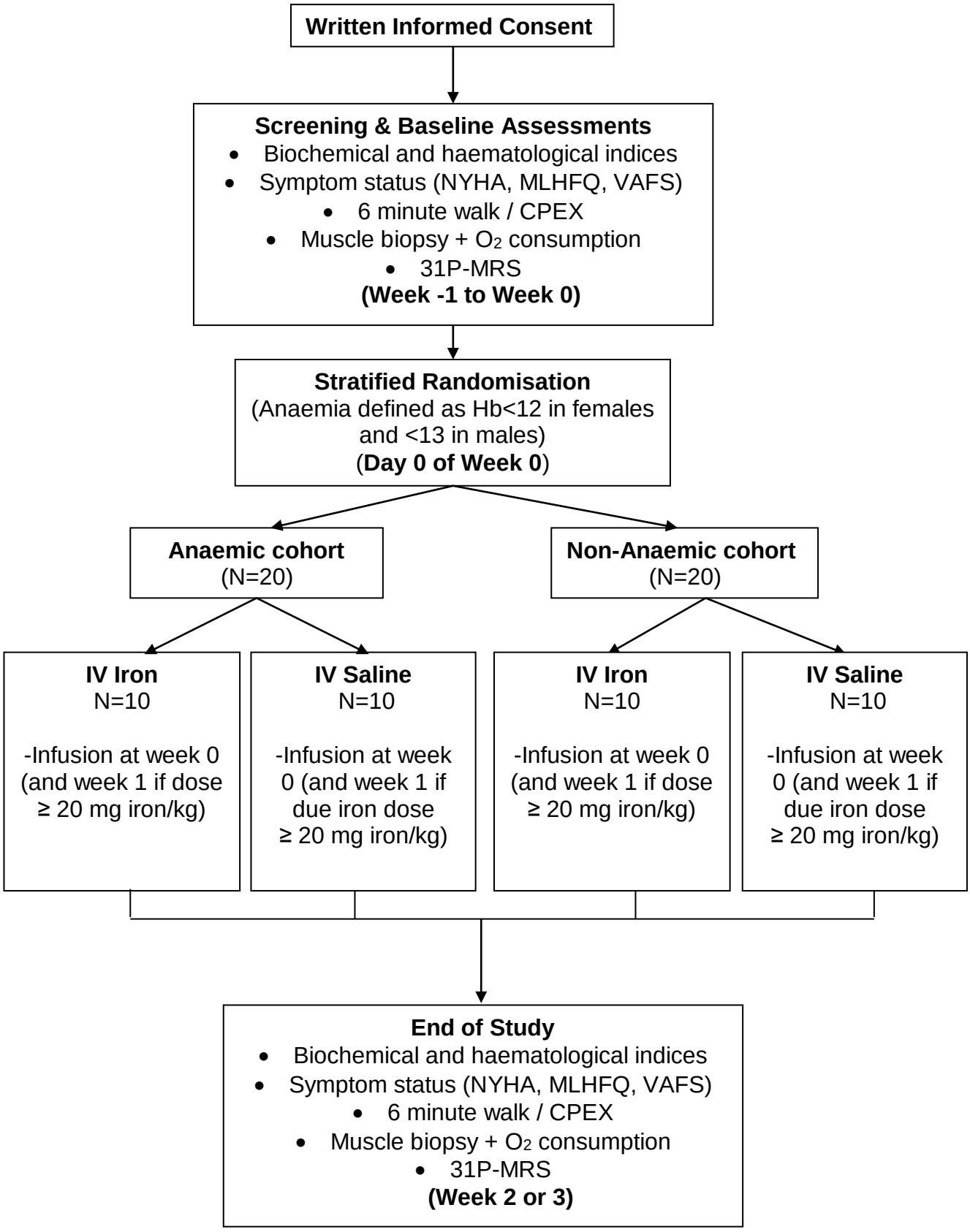
I attest to the accuracy of this report.

_____ _____

Chief Investigator **Date**

Print name

APPENDIX 1: STUDY DESIGN AND TREATMENT SCHEMA

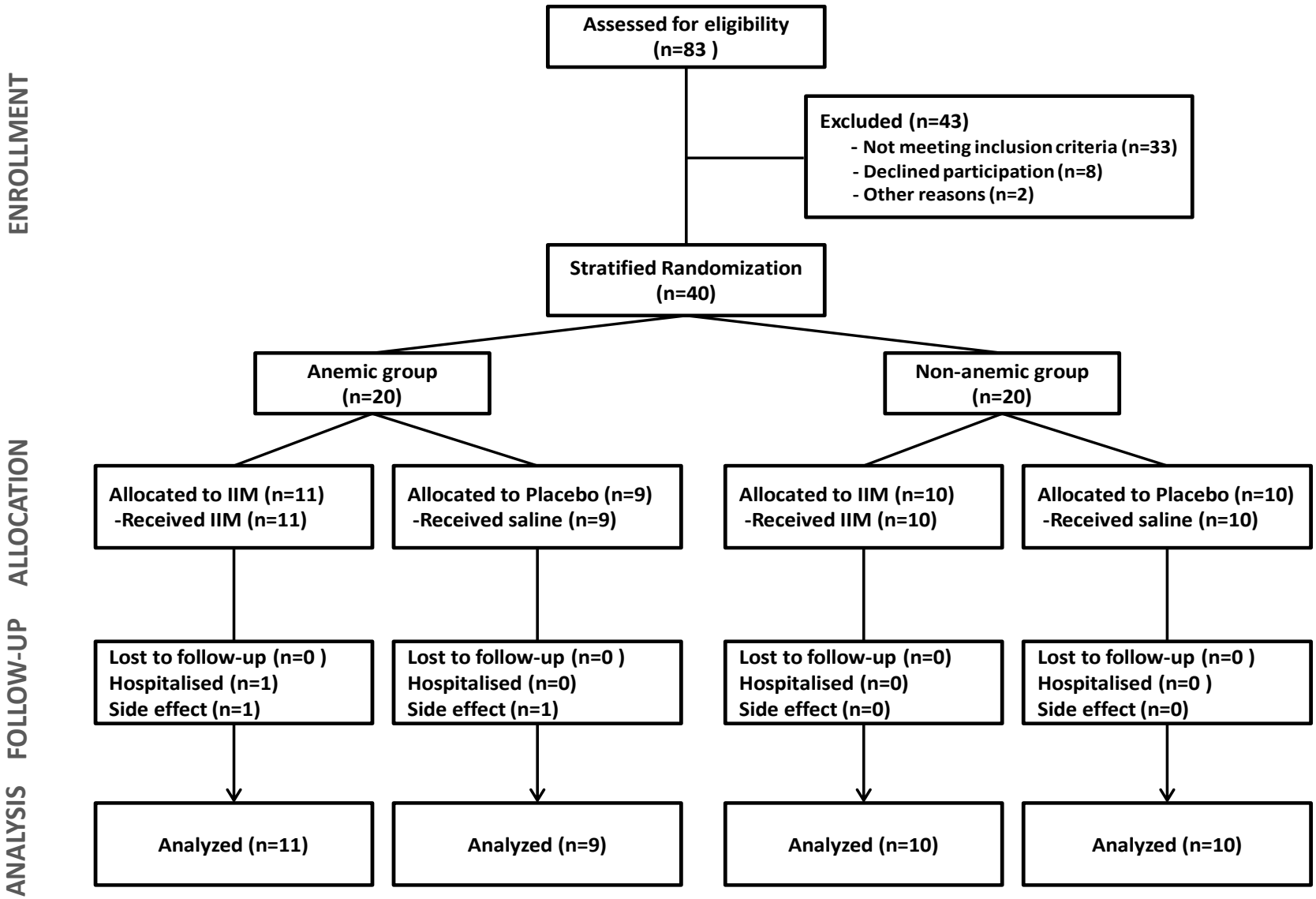


APPENDIX 2: SCHEDULE OF ASSESSMENTS

Tests & Procedures	Week -1	Week 0		Week 1*	Week 2 or 3	
	Day - 11 to -3	Day -2 to -1	Day 0	Day 7-10	Day 14-17 or 21-24	Day15-18 or 22-25
	Screening	Baseline Assessments	Baseline Assessments & Treatment	± Treatment	End of Study Visit 1	End of Study Visit 2
Informed consent	X					
Medical + drug history	X	X	X	X	X	X
Physical exam + BP/HR	X	X	X	X	X	X
Pregnancy test†	X					
FBC	X	X			X	
U+E, LFT, CRP	X	X			X	
SI,TIBC, Ferritin, TSAT	X	X			X	
TF, sTFr, BNP,cytokines		X			X	
Folate, Vitamin B12	X					
NYHA	X	X			X	
Echocardiography		X			X	
6 minute walk test		X			X	
CPEX		X			X	
KCCQ, VAFS		X			X	
31P-MRS			X			X
Muscle biopsy			X			X
IV Iron or Saline			X	X		
Adverse Events			X	X	X	X

BP= Blood pressure; HR= Heart rate; U+E= Urea & electrolytes; LFT= Liver function tests; CRP= C-reactive protein; SI= Serum iron; TIBC= Total iron binding capacity; TSAT= Transferrin saturation; TF= Transferrin; sTFr = Soluble transferrin receptor; NYHA= New York heart association; MLFHQ= Minnesota living with heart failure questionnaire;VAFS= Visual analogue fatigue score; NT-BNP= N-terminal brain natriuretic peptide; CPEX= Cardiopulmonary exercise test; 31P-MRS=31 phosphorus magnetic resonance spectroscopy; *only for patients whose calculated iron dose exceeds 20 mg/kg;† only for women of childbearing potential.

APPENDIX 3: FERRIC HF II CONSORT DIAGRAM



APPENDIX 4: PROTOCOL DEVIATIONS

Type of deviation	Number of subjects with deviations
Inclusion/exclusion criteria not met due to high folate or Vitamin B12 levels, erroneous GP diagnosis of asthma or LVEF 45.1%	4
Visit outside of window	4
Screening number 49 and Randomisation number 9 not allocated in error	n/a
Incorrect prescription	13
Missing endpoints due to equipment failure, logistical issues, normal physiology (e.g. irregular heart rhythm) or patient refusal	40
Patient randomised down wrong haemoglobin strata	1
Patient unable to attend end of study visit 1	1
Physicial examination (JVP) not collected in error	1

APPENDIX 5: BASELINE CHARACTERISTICS

	All patients		Anemic patients		Nonanemic patients	
	Placebo (n=19)	Iron Isomaltoside (n=21)	Placebo (n=9)	Iron Isomaltoside (n=11)	Placebo (n=10)	Iron Isomaltoside (n=10)
Demographics						
Age, years	62±13	70±12	63±15	74±6	61±11	65±15
Male gender, n (%)	13(68)	16(76)	7(78)	11(100)	6(60)	5(50)
Caucasian ethnicity, n (%)	14(74)	17(81)	6(67)	10(91)	8(80)	7(70)
Body mass index, kg/m^2	30±7	29±4	29±6	29±4	30±8	29±5
Ischaemic etiology, n (%)	10(53)	11(52)	6(67)	7(64)	4(40)	4(40)
Comorbidities						
Coronary artery disease	11(58)	13(62)	6(67)	9(82)	5(50)	4(40)
Hypertension	13(64)	13(62)	5(56)	8(73)	8(80)	5(50)
Hyperlipidemia	7(37)	7(33)	2(22)	6(55)	5(50)	1(10)
Diabetes mellitus	10(53)	10(48)	6(67)	7(64)	4(40)	3(30)
Atrial fibrillation/flutter	4(21)	6(29)	2(22)	3(27)	2(20)	3(30)
Clinical and quality of life						
LV ejection fraction, %	37±8	37±8	37±8	36±9	37±7	39±7
NYHA class	2.4±0.5	2.5±0.5	2.5±0.5	2.4±0.5	2.5±0.5	2.4±0.5
NYHA class III, n (%)	10(53)	9(43)	4(44)	6(55)	6(60)	3(30)
Systolic BP, mm Hg	122±17	124±16	115±14	125±14	128±17	124±18
Diastolic BP, mm Hg	71±14	73±10	67±11	71±8	75±16	76±12
Heart rate, beats/min	71±11	72±10	67±6	71±6	74±13	72±13
Respiratory rate, breaths/min	16±1	16±1	16±1	16±1	16±1	15±1
KCCQ overall score	53±20	64±23	58±27	66±19	48±13	62±28
Exercise parameters						
Peak VO_2, mL/kg/min	14.3±3.1	15.8±4.3	12.7±3.2	16.0±5.6	15.8±2.2	15.6±2.5
Peak respiratory exchange ratio	1.06±0.11	1.10±0.10	1.05±0.10	1.14±0.10	1.07±0.12	1.07±0.10
CPET exercise duration, s	582±202	627±241	551±252	636±289	610±153	617±191
VE/VCO_2 slope	31±5	38±10	31±5	43±12	31±5	34±7
6 min walking distance, m	313±67	324±79	295±70	299±84	330±64	351±68
Pre-exercise Borg dyspnea score	11±1	11±1	11±1	11±2	11±1	11±1
Post-exercise Borg dyspnea score	15±2	14±3	15±2	15±3	14±2	14±2

Data are mean±SD, numbers(%), or median (interquartile range).

APPENDIX 5: BASELINE CHARACTERISTICS (CONTINUED)

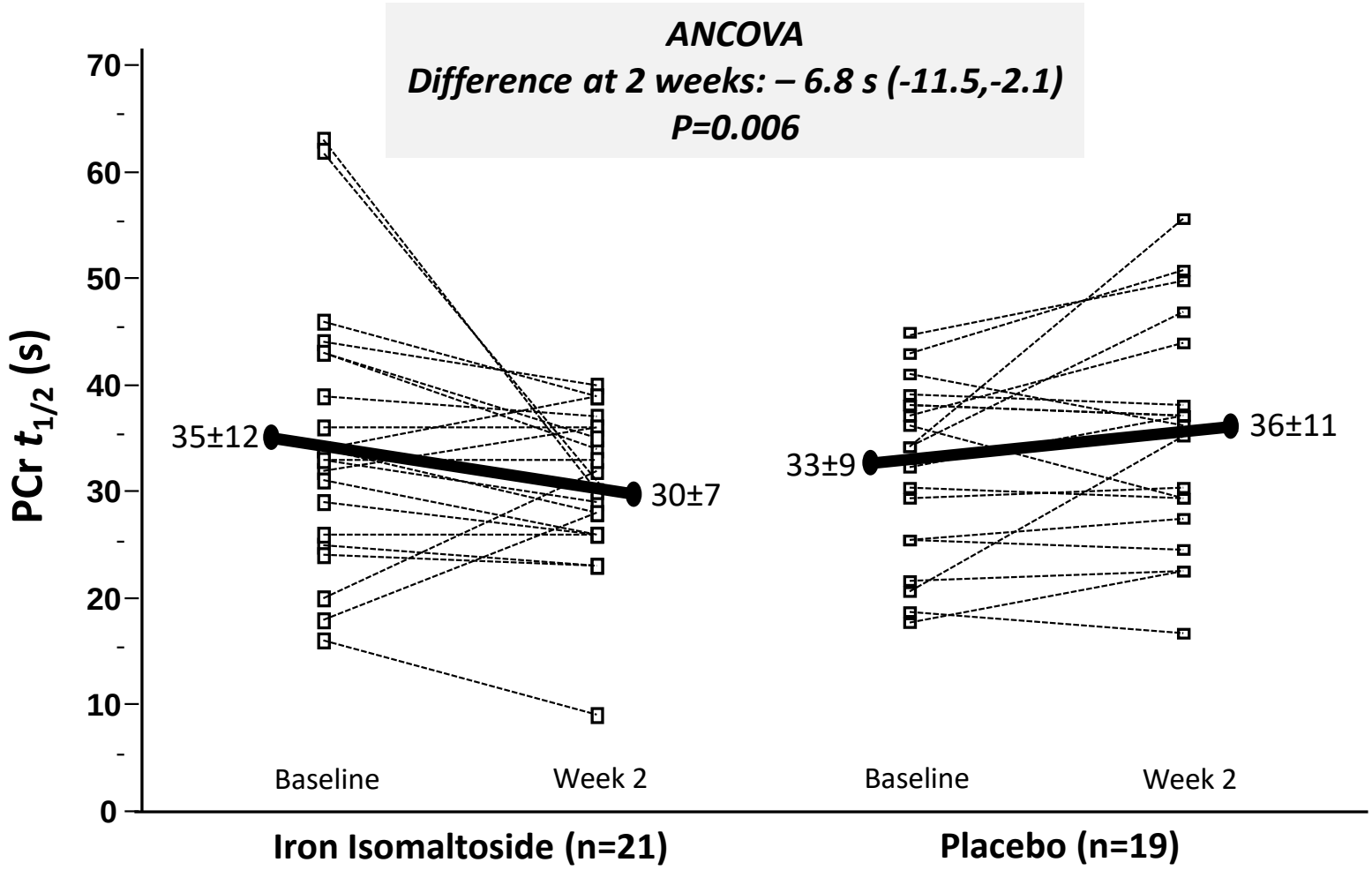
	All patients		Anemic patients		Nonanemic patients	
	Placebo (n=19)	Iron Isomaltoside (n=21)	Placebo (n=9)	Iron Isomaltoside (n=11)	Placebo (n=10)	Iron Isomaltoside (n=10)
^{31}P MRS measurements						
Resting PCr, mM	39±4	40±6	39±4	42±7	40±5	38±5
Exercise PCr, mM	26±4	25±6	23±5	25±7	28±3	25±5
Resting Pi, mM	4.3±1.0	4.0±0.7	4.4±1.0	3.9±0.8	4.1±1.1	4.2±0.6
Exercise Pi, mM	18.3±6.8	19.1±7.2	18.8±6.9	20.7±8.8	17.9±7.1	17.3±4.8
Resting pH	7.01±0.04	7.02±0.03	7.01±0.02	7.02±0.02	7.01±0.06	7.01±0.03
Exercise pH	7.00±0.05	6.96±0.10	7.00±0.04	6.97±0.07	7.00±0.06	6.95±0.13
Resting ADP, µM	8±5	8±6	8±6	7±4	8±5	10±8
Exercise ADP, µM	34±12	38±21	40±15	40±26	28±5	35±15
ADP $t_{1/2}$, s	23±6	25±9	24±6	26±12	23±6	24±4
PCr $t_{1/2}$, s	33±9	35±12	36±8	38±14	29±8	31±9
Laboratory measurements						
Ferritin, ng/mL	59(39-79)	34(18-50)	45(26-64)	33(18-48)	77(64-90)	44(28-60)
Transferrin saturation, %	18±10	21±8	12±5	16±7	24±10	25±6
Soluble transferrin receptor, mg/L	4.0±1.5	3.6±0.8	4.6±1.5	3.9±0.6	3.4±1.3	3.4±0.9
Hemoglobin, g/L	128±20	130±15	114±19	119±8	140±11	142±10
Creatinine, µmol/L	108±34	121±39	129±32	138±21	89±24	103±46
Aspartate transaminase, iU/L	22±9	22±8	25±8	21±7	20±10	24±8
C-reactive protein, mg/L	5(1-10)	6(3-9)	12(2-22)	5(3-8)	3(1-6)	6(1-11)
NT-proBNP, pg/mL	462(206-855)	1486(245-2054)	790(291-1944)	2696(423-3907)	316(133-629)	696(245-1900)
Treatment						
Diuretics, n (%)	12(63)	14(67)	8(89)	7(64)	4(40)	7(70)
ACE-inhibitor or ARB, n (%)	17(89)	16(76)	7(78)	9(82)	10(100)	7(70)
Beta-blockers, n (%)	16(84)	18(86)	7(78)	10(91)	9(90)	8(80)
Spironolactone, n (%)	12(63)	12(57)	5(56)	5(45)	7(70)	7(70)
Digoxin, n (%)	4(21)	6(29)	3(33)	2(18)	1(10)	4(40)
Anticoagulants, n (%)	3(16)	6(29)	1(11)	3(27)	2(20)	3(30)
Antiplatelets, n (%)	13(68)	13(62)	5(56)	8(73)	8(80)	5(50)

Data are mean±SD, numbers (%), or median (interquartile range).

APPENDIX 6: ENDPOINTS FOR TOTAL POPULATION

	Placebo (n=19)	Iron Isomaltoside (n=21)	Difference (95% CI)	ANCOVA P-value
Primary endpoint				
PCr $t_{1/2}$, s	36±11	30±7	-6.8(-11.5,-2.1)	**0.006**
Secondary endpoints				
ADP $t_{1/2}$, s	24±9	20±6	-5.3(-9.7,-0.9)	**0.02**
Hemoglobin, g/L	127±14	130±13	2.4(-3.5,8.4)	0.41
Ferritin, ng/mL	57(41-84)	369(232-495)	304(217,391)	**<0.0001**
Transferrin saturation, %	21±9	29±6	6.8(2.7,10.8)	**0.002**
NYHA class	2.6±0.5	2.3±0.5	-0.23(-0.46,-0.01)	**0.04**
6 min walking distance, m	324±67	347±72	15(-10,40)	0.24
Pre-exercise Borg dyspnea score	8±2	7±1	-0.6(-1.5,0.2)	0.12
Post-exercise Borg dyspnea score	15±2	12±3	-2.0(-3.7,-0.3)	**0.02**
Peak VO_2/kg, mL/kg/min	14.9±3.5	16.8±4.7	0.5(-1.0,1.9)	0.54
KCCQ overall score	55±24	68±17	12.7(-7.7,33.2)	0.18
LV ejection fraction, %	39±8	41±7	2.2(-1.1,5.6)	0.19
NT-proBNP, pg/mL	334(180-827)	1623(281-2453)	289(-461,1040)	0.44
Resting PCr, mM	40±5	40±6	-0.2(-3.5,3.0)	0.89
Exercise PCr, mM	26±6	25±6	-0.3(-3.6,3.0)	0.86
Resting Pi, mM	3.8±1.1	4.0±1.0	0.3(-0.3,0.8)	0.33
Exercise Pi, mM	18.2±7.2	17.6±6.7	-0.9(-5.0,3.2)	0.66
Resting pH	7.01±0.3	7.01±0.3	-0.01(-0.02,0.01)	0.44
Exercise pH	6.99±0.06	6.97±0.07	0(-0.04,0.04)	0.97
Resting ADP, µM	7±5	8±5	0.9(-2.0,3.8)	0.52
Exercise ADP, µM	34±15	37±18	1.2(-8.1,10.5)	0.80
Safety endpoints				
Systolic blood pressure, mm Hg	119±14	127±12	7.1(-0.3,14.5)	0.06
Diastolic blood pressure, mm Hg	72±13	74±10	0.7(-5.5,6.8)	0.83
Heart rate, beats/min	74±12	71±10	-2.9(-8.8,3.1)	0.34
Respiratory rate, breaths/min	16±1	15±1	-0.7(-1.2,-0.2)	**0.009**
Creatinine, µmol/L	103±38	106±41	-3(-26,20)	0.80
Aspartate transaminase, iU/L	22±6	30±26	8(-4,21)	0.21
C-reactive protein, mg/L	4(2-8)	6(2-11)	0.4(-3.0,3.9)	0.79

APPENDIX 7: INDIVIDUAL CHANGES IN PRIMARY ENDPOINT (PCr $t_{1/2}$)



APPENDIX 8: ANAEMIC AND NON-ANAEMIC SUBGROUP ANALYSES

	Placebo	Iron Isomaltoside	Difference (95% CI)	ANCOVA P-value
Anaemic patients	*n=9*	*n=11*		
PCr $t_{1/2}$, s	38±12	30±9	-8.4(-16.7,-0.2)	**0.04**
ADP $t_{1/2}$, s	24±9	18±7	-6.3(-13.5,0.9)	0.08
Hemoglobin, g/dL	117±12	124±6	5.5(-2.7,13.8)	0.18
Ferritin, ng/mL	45±25	456±161	413(295,530)	**<0.0001**
NYHA class	2.7±0.5	2.5±0.5	-0.3(-0.6,0.1)	0.09
Non-anaemic patients	*n=10*	*n=10*		
PCr $t_{1/2}$, s	35±11	31±5	-5.2(-10.6,0.2)	0.06
ADP $t_{1/2}$, s	24±9	21±5	-4.4(-9.6,0.9)	0.10
Hemoglobin, g/dL	135±9	137±15	0.5(-8.3,9.3)	0.72
Ferritin, ng/mL	67±24	274±149	176(68,284)	**0.003**
NYHA class	2.6±0.5	2.2±0.4	-0.2(-0.6,0.1)	0.23